04.14.16 Generating glycerol stock of pIB184

Made with Benchling

Project: McMaster iGEM '16

Authors: Mobeen Mubasher

Date: 2016-04-22

FRIDAY, 4/22/16

Entry by: Vivian Lau

Purpose:

To generate glycerol stock for pIB184 E.coli and miniprep remaining culture to obtain pIB184

Lab Notebook References:

N/A

Reagents:

Invitrogen PureLink Miniprep kit

Procedures:

1. Received ~7 mL culture of pIB184 in E. coli from Mahony lab, transported by Karanbir from St Joe's.
2. Generated glycerol stock -- ? Details (Karanbir)
3. Miniprepped plasmid (Vivian):
    a. Spun down culture at 3000 rpm for 15 mins
    b. Resuspended in 500 uL R3 buffer and split into two 1.5 mL Eppies
    c. Lysed and neutralized with L7, N9 respectively (5 min lyse)
    d. Spun down lysed bacteria 15,000 rpm for 10 mins @ RT
    e. Transferred supernatant to miniprep column
    f. Warmed TE to 37C on heat block
    g. Spun down columns, washed membrane and dried column (3 steps)
    h. Added 30 uL warmed TE to columns
    i. Eluted into the same tube.
    j. Nanodropped for concentration:
        I. 0.745ug/uL, A260 = 1.65, A260/280 = 1.62, A260/230 = 0.856
    k. Stored plasmid @ -20C until transport back to BSB lab.

04.22.16 Making LB stocks and Agar Plates (Amp/Kan/Cm)

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-04-22

FRIDAY, 4/22/16

Entry by: Vivian Lau

Purpose:

To generate autoclaved stocks of LB, ddH2O and LB agar

Inventory the lab space and purchase new items for wet lab

Lab Notebook References:

N/A

Reagents:

Ampicillin (200mg diluted previously in 10mL ddH2O), Gibco, 40mg/mL

Chloramphenicol

Kanamycin

LB Lennox (Bioshop?), 20g/L powder

LB Agar

Procedures:

Items generated:

Stock 1L bottle of LB

1. Diluted 20g of prepared powder in 1L of ddH2O
2. Inverted to dissolve powder.
3. Autoclaved liquid cycle for 45 mins (1:30h total time), Cycle 7

LB Agar (2x500mL)

1. Dissolved 16g of LB Agar mix into 500 mL ddH2O
2. Autoclaved liquid cycle as above.

Cm/Kan/Amp plates:

1. Split 500 mL bottle into ~350 mL and ~150mL kanamycin and chloramphenicol, respectively.
    a. Added 40 uL kanamycin (100mg/mL stock in ddH2O, -20C) to ~350 mL LB agar
    b. Added 200 uL chloramphenicol (100 mg/mL) stock to ~150 mL agar.
2. Poured 250 mL LB agar into separate sterile 500mL bottle and added 625 uL ampicillin (40mg/mL)
3. Poured ~1 sleeve kanamycin, ~8 chloramphenicol and ~1 sleeve ampicillin plates.

Legend for plates:

2 black lines = kan

1 black line = amp

1 red line = cm

2 red lines = ery

04.25.16 Reconstituting and transforming 2015 Kit Plate DNA into DH5a (1-4I, 1-14P, 2-4J and 2-6H)

Made with Benchling

Project: McMaster iGEM '16

Authors: Vivian

Date: 2016-04-25

MONDAY, 4/25/16

Entry by: Vivian Lau

Purpose:

To reconstitute DNA from 2015 Biobrick Distribution Plates and transform E. coli DH5a for plating on agar

Lab Notebook References:

N/A

Reagents:

Chloramphenicol LB agar plates

Invitrogen E. coli DH5a

Procedures:

1. Thawed 1 vial of 500uL DH5a from -80 on ice.
2. Autoclaved blue cell spreaders for plating (Cycle 1, 15 mins)
3. Aliquoted cells to 50 uL per 1.5 mL Eppendorf tube (Sterile) and placed unused vials back into -80C
4. Reconstituted 1-4I, 1-14P, 2-4J and 2-6H wells from 2015 Distribution Plates with 10uL sterile ddH2O.
5. Added 1 uL of DNA to 50 uL of chemically competent cells, incubated on ice for 10 mins for complexing.
6. Heat shocked 4 samples in 42C waterbath for 1 min.
7. Added 900 uL of SOC media to tubes, and attached to water bath shaker for recovery @ 37C for 1 hr.
8. Plated 150 uL of bacteria on chloramphenicol agar plates and placed in 37C incubator O/N.

Notes:

First set of transformations were scrapped - accidentally added SOC before heatshocking.

04.26.16 Liquid culture prep and more DNA reconstitution

Made with Benchling

Project: McMaster iGEM '16

Authors: Vivian

Date: 2016-04-26

TUESDAY, 4/26/16

Entry by: Vivian Lau

Purpose:

To reconstitute more DNA from 2015 Biobrick Distribution Plates and transform E. coli DH5a for plating on agar

To start liquid cultures from colonies obtained from transformations the day before

Lab Notebook References:

04.25.16 Reconstituting and transforming 2015 Kit Plate DNA into DH5a

Reagents:

Chloramphenicol LB agar plates

Invitrogen E. coli DH5a

Procedures:

1. Thawed 3 vials of 50uL DH5a aliquots from -80 on ice.
2. Reconstituted 2-18D, 3-4O, 4-7P wells from 2015 Distribution Plates with 10uL sterile ddH2O.
3. Added 1 uL of DNA to 50 uL of chemically competent cells, incubated on ice for 10 mins for complexing.
4. Heat shocked 3 samples in 42C waterbath for 1 min. (RB)
5. Added 900 uL of SOC media to tubes, and attached to water bath shaker for recovery @ 37C for 1 hr. (RB)
6. Plated 250 uL of bacteria on chloramphenicol agar plates and placed in 37C incubator O/N. (RB)
7. Re-plated 300uL of 1-14P from yesterday's transformation on fresh CmR plate - no colonies formed on yesterday's plate.
8. Picked 1 colony from each of 3 plates previous day and made 3 LB overnights (cmR diluted to 100ug/mL from 30 mg/mL stock)
9. Incubated in shaker (225rpm) @ 37C O/N.

Notes:

04.27.16 Molbio Workshop Day 1

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-04-27

WEDNESDAY, 4/27/16

Entry by: Vivian Lau, Dhany, Max, Mobeen, Rayu, Tony, Fawad (Mohammed)

Purpose:

Lab Notebook References:

04.25.16 Reconstituting and transforming 2015 Kit Plate DNA into DH5a

Reagents:

Chloramphenicol LB agar plates

30mg/mL chloramphenicol

LB Lennox liquid media

Procedures:

Overnight liquid cultures for 3-4O, 2-18D, and 1-14P

1. Added 40uL of 30mg/mL chloramphenicol to 12mL of LB Lennox liquid media
2. Aliquoted 4mL of this mixture to three separate 14mL culture tubes
3. Added a single colony of of either 3-4O, 2-18D, or 1-14P into each tube to make three separate overnight liquid cultures
4. Liquid cultures will be stored overnight at 37°C

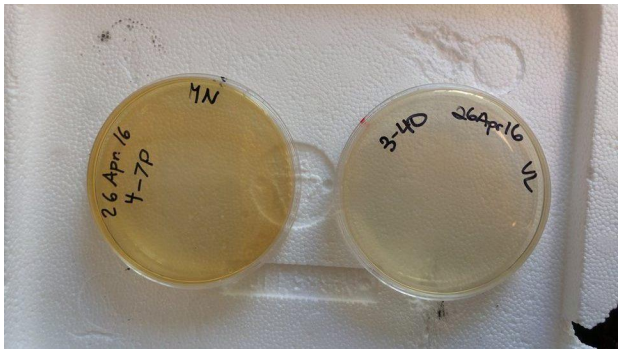
Digestions for 2-4J, 1-4I, 2-18D

1. Added 5 uL DNA, 1 uL 10x cutsmart, 1 uL SpeI, 1 uL EcoRI, 2 uL ddH20 to 1.5 mL microfuge tube.
2. Put in water bath for 30 minutes.
3. Digestions were run on gel (see below).

Notes:

6. Image of the two re-plated bacteria

4-7P_3-4O_plates_27-04-2016.jpg



PCR

1. Dilute the primers from 10 uM to 200 nM
2. Once complete add **45 uL** of the Platinum PCR Supermix
3. Add 2.5 uL of the Forward primer and 2.5 uL of the Reverse primer
4. Finally add in 8uL of the template DNA (cDNA) (0.127 ug/uL)
5. Repeat this 2 more times

Once the PCR samples have been compiled, add them to the thermocycler with these varying temperatures in order to have them undergo PCR amplification and replication

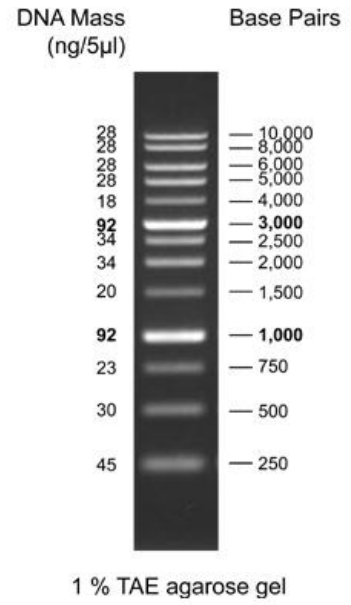
1. 58 Degrees Celsius
2. 55 Degrees Celsius
3. 53 Degrees Celsius

Agarose Gel Electrophoresis

1. Prepared 100mL 1% agarose gel with 4uL RedSafe
2. Added 1uL 10X DNA loading buffer to 10 uL SpeI/EcoRI double digests of 2-4J, 2-18D, 1-4I
3. Loaded wells with above samples (~11uL ea); ladder lane loaded with 10uL ladder. Loading order:
4. Ran at 100V for 20 mins in 1X TAE

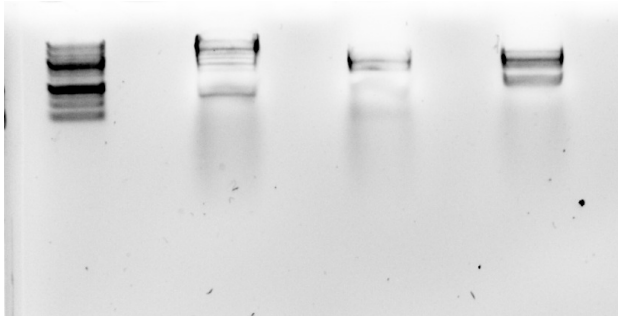
Loading order:
Lane 1 - 1kb ladder (GeneDireX)
Lane 3 - 2-4J digest
Lane 5 - 2-18D digest
Lane 7 - 1-4I digest

GeneDirex Ladder.jpg



1 % TAE agarose gel

Vivian 2016-04-27 16hr 13min.png



Gel Analysis:
Two strong bands in ladder correspond to 3kb and 1kb.
Lane 1 has a dropout fragment ~750bp, and lane 3 has a ~1kb dropout.

04.27.16 Miniprep of LasR, LuxR, mCherry (1-14P, 3-4O, 2-18D)

Made with Benchling

Project: McMaster iGEM '16

Authors: Mobeen Mubasher

Date: 2016-04-27

WEDNESDAY, 4/27/16

Notes

Entry by: Mobeen

Purpose:

Usage of Thermo Scientific Plasmid Miniprep kit to prepare plasmid DNA

Lab Notebook References:

N/A

Reagents:

1-14P: LasR/PAI1 Inducible Promoter + RBS (B0030) + RFP Constitutive Promoter + RBS (B0030) + LasR

3-40 (luxR)

2-18D (mcherry, RFP)

Procedures:

Plasmids for were above wells were mini prepped:

a. 250 uL of resuspension fluid added to micro centrifuge tube. Centrifuged for 5 minutes

b. 250 uL of Lysis solution was added and vials were inverted until solution became clear.

c. 350 uL of Neutralization solution was added to sample. Sample was centrifuged for 5 minutes.

d. Supernatant was transferred into spin columns and centrifuges for 1 minute

e. 500 uL of wash solution was added to spin column and centrifuged for 1 minute.

f. 50 uL of elution buffer was added to elute plasmid DNA.

g. Purified plasmid dna was stored at -20 degrees celsius

Notes:

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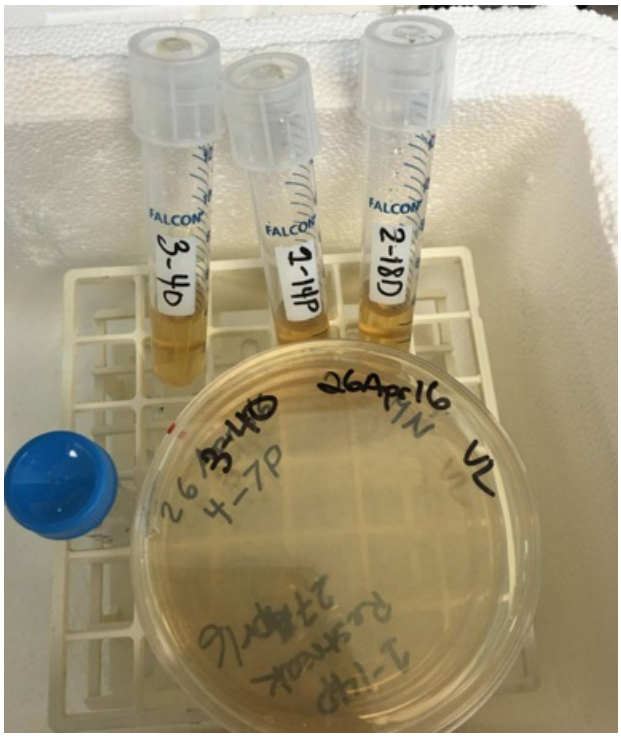

04.28.16 Molbio Workshop Day 2

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-04-28

THURSDAY, 4/28/16

Entry by: Vivian Lau, Rayu Bhatt, Yu Fei Xia, Clara Long, Yosef Ellenbogen

Purpose:

Lab Notebook References:

Reagents:

Chloramphenicol LB agar plates
Invitrogen E. coli DH5a
Invitrogen PureLink Miniprep kit
S.O.C. Medium

Procedures:

Transformations for 3-4O and 4-7P

1. E. coli DH5a was alquoted into 10 1.5mL centrifuge tubes containing 50uL each. 9 tubes were returned to storage at -80°C
2. 2uL of each of the 2 DNA were added to 2 samples tubes (one newly aliquoted, one from previously).
3. Incubated on ice for 10mins
4. heat shock for 1 min at 42°C
5. placed back on ice, 900uL of S.O.C. medium added to each sample.
6. Recovery: samples were placed in heat bath for 40mins with shaker on at 175rpm
7. the samples were plated, with 300uL on each plate.
8. stored at room temp for the weekend.

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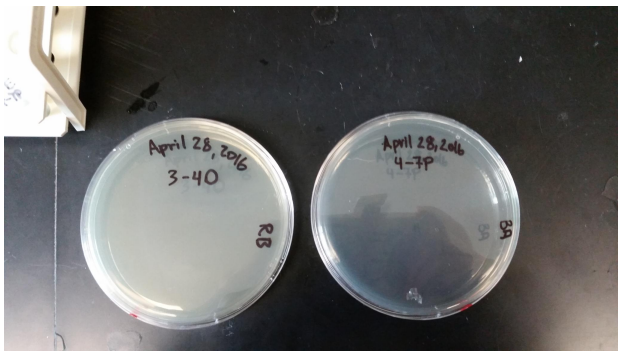

Agarose Gel Electrophoresis

1. Remaining 50mL of agarose gel prepared on April 27 was used, with the addition of 1.5uL RedSafe
2. Wells were loaded with 30 uL using one of three PCR samples, with each sample contributing to two wells

Loading order:
Lane 1: Ladder
Lane 2: Sample 1
Lane 3: Sample 1
Lane 4: Sample 2
Lane 5: Sample 3
Lane 6: Sample 3

Note: Some of Sample 2 was split during gel loading, and thus there was only one lane for Sample 2.

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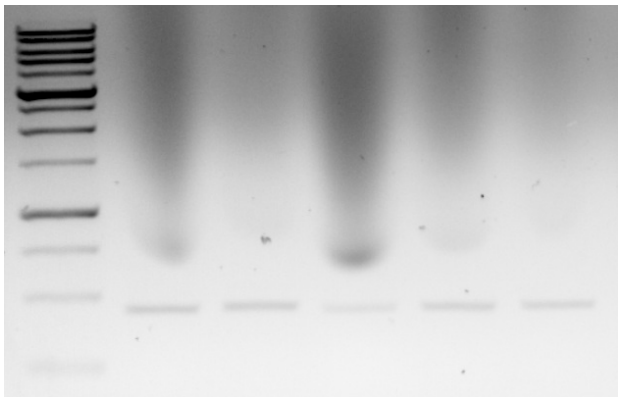


Gel analysis:

- The cause of the smear seen in all 5 sample lanes is unclear
- The 450bp band seen in all 5 sample lanes corresponded to IL-2

Gel Extraction

1. Gel was visualized under UV, and bands at ~450bp in lanes 2-6 were excised with a razer blade.
2. The cut gel pieces that contained DNA were pooled together and placed into two eppendorfs which were stored in fridge
3. The remainder of the gel was disposed of.
4. The rest of the dna extraction and purification from the cut gel pieces will be completed on Monday, May 2nd.

Notes:

1. Overnight liquid culture of 3-4O did not work

05.02.16 Liquid culture prep of 3-40 and 4-7P

Made with Benchling

Project: McMaster iGEM '16

Authors: Ramneet Mann

Date: 2016-05-02

MONDAY, 5/2/16

Entry by: Rayu Bhatt, Yosef Ellenbogen

Purpose:

To start liquid cultures from colonies obtained from transformations of 3-4O and 4-7P

Reagents:

Chloramphenicol (30mg/mL)

Transformed 3-4O and 4-7P colonies

LB Lennox liquid media

Procedures:

1. Pipetted 20mL of LB Lennox liquid media into two erlenmeyer flask
2. Added 40uL of chloramphenicol to each flask
3. Added a colony of either 3-4O or 4-7P to each flask
4. Sealed flasks with tin foil and placed in cell shaker overnight at 37°C at 200rpm
5. Placed remaining cell cultures into 4°C fridge for preservation

Notes:

1. Cultures of 3-4O yielded many colonies
2. Cultures of 4-7P yielded two visible colonies

05.03.16 Gel Extraction of IL-2 and Overnights of 3-40 and 4-7P

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-03

TUESDAY, 5/3/16

Entry by: Ramneet Mann

Purpose: To do DNA Gel Extraction of IL-2 and prepare overnight cultures of 3-40 and 4-7P

Reagents:

- The buffer solution reagents were provided in the gel extraction kit
- 100 mL of 95% Ethanol
- 3-40
- 4-7P
- LB Lennox liquid media
- Chloramphenicol
- EcoRI
- Pst1
- plB184 and 2-18D (mChery)

Gel Extraction Procedure:

1. Added 200 uL of extraction buffer to both IL-2 gel extract tubes present.
2. Heated water bath to 50 °C, incubated gel mixture for approimately 10 minutes
3. Added 200 uL of Ethanol 95% to both tubes of Il-2 gel extract.
4. Added extra 200 uL of extraction buffer to 1 of the 2 IL-2 tubes (labelled #1), re-incubated in water bath at 50 °C for approximately 10 minutes, and added an extra 200 uL of 95% Ethanol.
5. Centrifuge IL-2 gel extract #1 mixture in DNA purification Micro Column at 13, 000 RPM for approximately 80 seconds. Solution failed to flow through.
6. Weighed and estimated approximately 500 mg of agarose gel for IL-2 gel extract tube #2
7. Added an extra 300 uL of extraction buffer to extract #2. for total of 500uL buffer, incubated at 65 °C for approximately 10 minutes, and added 300 uL of Ethanol, for a total of 500uL ethanol.
8. Centrifuged IL-2 gel extract tube #2 for 2 minutes, poured flow through into waste.
9. Added 200 uL of pre-wash buffer to IL-2 gel extract tube #2, centrifuged for 2 minutes, and poured flow through into waste.
10. Added 700 uL of wash buffer to IL-2 gel extract tube #2 and centrifuged for 2 minutes. This step was repeated upon completion.
11. The empty DNA Micro Column was centrifuged for 2 mins.
12. THe DNA Micro Column was transferred to a clean 1.5 mL microcentrifuge tube, and 10 uL of Elution Buffer was added to the micro column, followed by centrifuging for 1 minute.
13. DNA from the micro column labelled IL-2 DNA Gel Ext was stored at -20 °C.

Preparing Overnights Procedure:

1. Added LB Lennox liquid media and Chloramphenicol into two separate flasks, and colonies from 3-4O and 4-7P were added to their respective flasks using a tooth pick

Digestion Procedure:

1. Made 1% agarose gel with 1X RedSafe
2. 5uL plB184 and 2-18D (mCherry) were digested in a 10uL solution with 2U EcoRI-HF and 2U PstI-HF in 1X cutsmart (ddH2O to dilute to appropriate volume and conc.)
3. 1uL 10X DNA loading buffer added to digests

4. 11uL sample loaded onto agarose gels, with 10uL 1kB DNA ladder
5. Gel run at 100V for ~30mins (see **Fig. 1**)
6. Gel slices of isolated mCherry (270mg) and linearized pIB184 (190mg) were cut. (Bands selected marked on **Fig. 1**). Stored at room temp

Notes:

- Approximately 100 mL of 95% Ethanol was obtained from stock and labelled
- The extra step 4 in the Gel Extraction Procedure was completed because the fluid appeared very viscous and "gel-like", and it was not in a state to be centrifuged
- After centrifuging in step 5, despite the extra step 4, there was no flow through in the column as there was a gel like substance lodged in the filter and blocking filtering from occuring - the rest of the steps are therefore completed with IL-2 gel extract tube #2
- After estimating the agarose gel in step 6, step 7 was completed to maintain a 1:1 ratio of the gel and buffer solutions added, and to ensure the gel was adequately dissolved to allow centrifugation to be successful

05.04.16 Miniprep, Glycerol Stock, Gel Extraction, Ligation, Transformation (if time)

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-05-04

WEDNESDAY, 5/4/16

Entry by: Fei Fei, Yosef, Tony, Melodie, Mobeen

Purpose: To miniprep overnight cultures of 3-4O, prepare a glycerol stock of 3-4O, gel extract 2-18D and linearized pIB184

Reagents:
Invitrogen Miniprep Kit

Miniprep

1. 4 tubes of 400uL 3-4O culture each were pelleted (pellet of 200uL twice)
2. the invitrogen miniprep protocol was followed (refer to previous entries for details)
3. 4 1.5mL sample tubes were stored at -20degrees.

Notes: 4-7P did not grow overnight. There are no more cultures to pick on the plate. Possibly used a plate with the wrong resistance. **4-7P needs to be re-transformed.** Also, the lysate solution for one of the miniprep kits ran out, use the other kit. **The miniprepped DNA samples need to be nanodropped for concentration**

Glycerol Stock

1. 500uL of the 3-4O culture was aliquoted and placed in a screw top tube.
2. tube was placed in the 4degree fridge, to be continued by VIvian tonight.

Gel Extraction

Note: newer extraction kit was used; old one ran out of columns so protocols differ from may 3

1. Added 1:1 v/w binding buffer to mCherry (270uL) and pIB184 (190uL) gel slices
2. Incubated 10mins @ 58C, inverted to mix throughout
3. Mixed by pipetting and transferred to column; centrifuged 1 min; flowthrough discarded
4. Added 100uL binding buffer; centrifuged 1 min; flowthrough discarded
5. Added 700uL wash buffer; centrifuged 1 min; flowthrough discarded
6. Centrifuged 1 min; flowthrough discarded
7. Added 50uL elution buffer, centrifuged 1 min; flowthrough collected
8. Stored @ -20C. Labels: mCherry DNA, pIB184 Linearized.

Ligation

1. 20uL reaction of 2uL ligation buffer, 3uL linearized pIB184, 14uL mCherry DNA, 1uL T4 Ligase
2. Incubated overnight at room temperature

Transformation

1. Added 1ul of 4-7P (from -20 freezer) into 50ul of DH5a chemically competent cells
2. incubated for 10 minutes on ice for cells to complex with DNA
3. heat shocked at 42 degrees for 42 seconds
4. plated on amp^r plate and placed in the 37 degree incubator
5. tomorrow will be doing overnight culture

Digestion Gel Electrophoresis

1. made 1% agarose gel
2. loaded 2-18D (mCherry) that was digested with EcorI-hf PstI-hf (done yesterday as well) in order to obtain more DNA so that more ligations can be done with mCherry into pIB184
3. no picture was taken, however the gel piece was cut and placed in 4 degree fridge to do extraction tomorrow

05.05.16 Transformation of 4-7P into SE E. Coli

Made with Benchling

Project: McMaster iGEM '16

Authors: kbrar4013

Date: 2016-05-05

THURSDAY, 5/5/16

Transformed 4-7P plasmid construct from iGEM (resuspended in 10uL h20 from before) carrying LuxR (pSB1A2 backbone) into SE E. coli DH5a.

Protocol changes:

- Incubated 50ul of transformant with 3ul of DNA (200-300pg/uL)
- Added 900uL of SOC and incubated at 37 degrees without shaking for 45 minutes (there was shaking for approximately 15 minutes, but we realized the tube wasn't fully in the water bath and had to turn off shaking so that the entire tube could be submerged for the rest of the time) prior to plating
- Spun down culture at 12,000rpm for 2 minutes and resuspended cell pellet in 250uL (removed 700uL)
- Plated both the 250uL concetrated transformants as well as the 700uL supernatant on AmpR LB agar plates at 37 degrees and 5% CO2 overnight

05.06.16 Gel Extraction of mCherry (2-18D)

Made with Benchling

Project: McMaster iGEM '16

Authors: Melodie Kim

Date: 2016-05-06

FRIDAY, 5/6/16

Entry By: Rayu Bhatt, Melodie Kim

Purpose: To extract mCherry DNA from gel

Reagents and Procedure: Refer to Gel Extraction Kit, 95% Ethanol

Gel Extraction

1. Added 1:1 v/w binding buffer to mCherry (200uL)
2. Incubated 10mins @ 55C, inverted to mix throughout
3. Mixed by pipetting and transferred to column; centrifuged 2 min; flowthrough discarded
4. Added 100uL binding buffer; centrifuged 1 min; flowthrough discarded
5. Added 700uL wash buffer; centrifuged 1 min; flowthrough discarded
6. Centrifuged 1 min; flowthrough discarded
7. Added 10uL elution buffer, centrifuged 1 min; flowthrough collected
8. Stored @ -20C. Labels: mCherry

05.10.16 Reconstituting and transforming 3-9N, 3-6M, making Ery agar plates

Made with Benchling

Project: McMaster iGEM '16

Authors: Vivian

Date: 2016-05-10

TUESDAY, 5/10/16

Entry By: Fei Fei, Vivian

Purpose: To reconstitute and transform 3-9N, 3-6M, and make erythromycin agar plates (2 red lines)

Reagents and Procedure:

DH5a E coli
sterile H2O
2015 iGEM kit plate 3
SOC medium

Reconstitution: (Fei Fei)

1. hole was poked in appropriate spots on the 2015 iGEM kit plate 3 with a pipet tip
2. 10uL of sterile water was added to each, and mixed.
3. Waited 5mins for DNA to fully dissolve
4. transferred the DNA to labeled eppendorfs, stored in -20degrees freezer

Transformation: (Fei Fei)

1. 2 samples of 50uL SE cells were thawed on ice
2. 2uL DNA of 3-9N and 3-6M were added to each sample of cells
3. incubated on ice for 10mins
4. heat shock 1min in 42degree water bath
5. 900uL SOC medium added to each sample
6. samples were take back to Vivian's lab to be placed at 37degrees for recovery

Making erythromycin (Em) plates and stock solution: (Vivian)

1. Calculated 10uM final dilution for E. coli cultures is ~7.34ug/mL (MW=733.93g/mol)
2. Created stock of 7.34mg/mL (0.074g powder) solution dissolved in 95% EtOH. Stored @ -20C.
3. Diluted 1:1000 (275uL) of Em into ~275mL remaining of agar, melted in the microwave or ~5 mins (2 min increments until melting thoroughly)
    a. Ensure agar is graspable by bare hands without being too hot for adding antibiotics
4. Poured ~12 Em agar plates - labelled with 2 red stripes.

05.11.16 Liquid culture of 3-9N (LasI), 3-6M (LasR), Ligation of pIB184-mCherry + ctrl (linearized plasmid), transformation of pIB184-mCherry into DH5a E. coli

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-05-12

THURSDAY, 5/12/16

Entry by: Yosef Ellenbogen, Rayu Bhatt

Purpose:

1. Set up liquid culture 3-9N, and 3-6M
2. Ligation of pIB184-mCherry + ctrl (linearized plasmid)
3. Transformation of pIB184-mCherry in DH5a chemically compoetent E. coli

Lab Notebook References:

N/A

Reagents:

Invitrogen PureLink Miniprep kit

Procedures:

1. Liquid culture
    a. 16 uL of 50mg/ml chloramphenicol into 8ml of media (LB Lennox)
    b. 4mL used for each culture
    c. One colony inocculated in lequid media of each 3-9N and 3-6M
    d. Cultured overnight at 37C in water bath shaking at 200rpm
2. Ligation
    a. Added 10uL mCherry, 2uL linearized pIB184, 1uL T4 ligase, 1.45uL ligase buffer.
    b. For control added 8uL linearized pIB184, 1uL T4 ligase, 1uL ligase buffer. - confirm?
    c. spun down for 2 minutes
    d. left at room temp overnight
3. Transformation
    a. Thawed S.E DH5a from -80 freezer on ice.
    b. Thawed ligation of mCherry-pIB184 ligation from last week found in -20 freezer
    c. aliquotted 1uL of ligation into 50uL aliquot of Dh5a.
    d. let sit for 10 minute to allow DNA to complex with cells
    e. heat shocked for 42 seconds at 42 degrees
    f. added 900uL of soc media to eppendorf and let sit on ice for 1hr30min
    g. Spun for 2 minutes and then dumped supernatant gently to ensure pellet remainds undisturbed.
    h. resuspended pellet in remaining soc that was present after dumping supernatant.
    i. Plated the ~50uL left in the eppendorf on erythromycin plate (two red lines) and placed in autoclave room overnight

05.12.16 - 3-9N, 3-6M miniprep + transformation of pIB184-mCherry

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-05-12

THURSDAY, 5/12/16

Entry by: Tony

Purpose:

1. Miniprep overnights of 3-9N, 3-6M
2. Transformtion of pIB184-mCherry into DH5a E.coli

Procedures:

Miniprep

1. Pelleted 4mL of overnight cultures 3-9N and 3-6M
2. Resuspended in 250uL resuspension buffer
3. Added 250uL lysis buffer, inverted to mix
4. Added 350uL neutralization buffer
5. Centrifuged 5 mins to pellet cell debris; transferred supernatant to column
6. Centrifuged 2 mins; flowthrough discarded
7. Added 500uL wash buffer, centrifuged 2 mins; flowthrough discarded
8. Repeated wash
9. Centrifuged 2 mins to remove remaining wash buffer; flowthrough discarded
10. Eluted with 50uL elution buffer into 1.5mL eppendorfs, centrifuged for 2 mins

Labels: 3-9N, **3-6H (Mislabel)**

Transformation

1. 1uL ligation (or control) was combined with 20uL DH5a
2. Complexed on ice for 20 mins
3. Heat shocked at 42C for 50s
4. Returned to ice for 2 mins
5. Added 300uL SOC, incubated at 37C with shaking for ~40 mins
6. Pelleted and resuspended to increase concentration
7. Plated onto Ery plates.

05.16.16 Running failed pIB184-mCherry ligations on a gel

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-17

TUESDAY, 5/17/16

Entry By: Yosef

Purpose: Troubleshooting step 1: To figure out whether the ligations had worked because the transformations have repeatedly not been working

Reagents and Procedure:
pIB184-mCherry Ligation May4
pIB184-mCherry Ligation May 11
pIB184 control May 11
pIB184 linearized
1% agarose gel

Gel electrophoresis:
Lane 1: 1kb ladder
lane 3: pIB184 ligation control (no insert) May 11
Lane 5: PIB184-mCherry Ligation May 11
Lane 7: pIB184-mCherry184 May 4
Lane 12: pIB184 linearized

- Gel was plased in ziploc bag and placed in 4 degree fridge

Conclusion:
- note: both the ligation and corresponding control from May 11 were left at room temp for entire weekend, and thus it is understandable that no prominant bands are seen at the expected ~6000kb for those lanes
- it seems that in lane 7 where pIB184-mCherry ligation (may 4) is loaded, there are two discernable bands indicating possibly that the ligation was sucessful and that the two bands correlate with supercoiled and relaxed plasmids. Additionally no dropout is seen at ~600bp which could mean that all of it was ligated into the backbone, however it is more likely that it is either at such low concentration that it is not visible (especially considering the mCherry was extracted from a gel containing an already faint band - see previous lab entry from May 03, 2016 entry). Another possibility is that no ligation occured but rather the two bands are linearized plasmid backbone and re-ligated plasmid backbone both of which are background. Ultimately, this was not entirely conclusive and we need to review ligations protocol more extensively.
- Lane 12 was loaded with linearized pIB184 backbone but nothing was seen, indicating either there was no plasmid, or that there was a loading error. The former is more likely. There is a confusing faint band at ~600kb which Yosef, Tony, and Max couldn't figure out what it could beunless the eppendorf was mislabelled and this was in fact purified mCherry.

05.18.16 Transformation of pIB184

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-18

WEDNESDAY, 5/18/16

Entry By: Max

Purpose: Transformation of pIB184

1. SE cells thaw on ice
2. Room temperature erythromycin agar plates
3. 1uL pIB184 DNA into 50uL SE cells
4. Eppendorfs on ice for 15min
5. Cleaned shaker (rust)
6. Added eppendorfs into waterbath at 42.3deg for 42sec
7. Iced cells for 2min
8. Added 900uL of SOC media
9. Centrifuged for 2min and removed supernatant
10. Spread on plates
11. Incubated in waterbath overnight

05.21.16 1-4I, 3-12K, 3-1B transformations, other gels and digests

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-20

FRIDAY, 5/20/16

Entry By: Max

Purpose: Preparation for Discovery Day and ncessesary genes

Failed Miniprep of 1-4I, 1-14P, 3-12K

No growth found, transformed again instead onto both plates and in flasks

1-14P was replaced with 3-2B (of the 2016 Kit) as 1-14P failed to grow in the past, and 3-2B has a similar size

Replace 1-4I reconstituted with 1-4I miniprepped DNA

Transformation of 1-4I (miniprepped), 3-12K (reconstituted), 3-1B (reconstituted)

1. Reconstitution of 3-2B from 2016 iGEM Kit
    a. 10uL added into well 3-12K
    b. Transfered into 1.5uL eppendorf
2. Transformation protocol
    a. Transfer of 1uL of each DNA sample to SE cell 50uL aliquot
    b. On ice for 10min to complex
    c. 42sec in 42deg bath
    d. 2min on ice
    e. Added 900uL of SOC media to each eppendorf
    f. Incubated 1.5h at 37deg
3. Preparation of liquid media (halted, alarm)
    a. Attempt to autoclave 100mL of LB taken from stock into two bottles (50mL LB in each = half-filled), with caps loosed, in catch tray
    b. Autoclave Alarm: Too Long in Break Time
    c. Waiting for the autoclave to complete the extended cycle
    d. Notified Dr. da Silva, Alison, and Alastair
4. Preparation of liquid media
    a. Transfered 20mL of LB from stock into each of 3 sterile 125mL Erlenmeyer flasks (autoclaved earlier in the morning)
    b. Added 24uL of 50mg/mL Chloramphenicol stock to each flask
5. Added 500uL of solution to erlenmeyer flask, for each gene
    a. Incubated overnight at 37deg in waterbath shaker in BSB207
6. Centrifuged the rest, removed supernatant, and plated on Cm plates
    a. Incubated overnight at 37deg in waterbath in Prep Lab

Digest mCherry, pIB184, and pUC19 with EcoRI

05.20.16 1-4I, 1-14P, 3-12K transformation and liquid culture; pUC19, pIB184, mCherry miniprep

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-20

FRIDAY, 5/20/16

Entry By: Max, Yosef

Purpose: Preparation for Discovery Day and necessary genes

Transformation:

2016 Plate Kit 3 - 12K

- Reconstituted with 10uL
- When red, moved to 1.5mL eppendorf

1-4I, 1-14P, 3-12K

- For those 3, 1uL of DNA added to 50uL of SE cells (taken from source)
- On ice for 10min to complex
- 42sec in 42deg bath
- **2min on ice --> shoudln't this be 10??? (Yosef) (this is what we did though for previous entries? -Max)**
- Added 900uL SOC media
- Incubated 1h at 37deg
- Added everything to erlenmeyer flask with 24uL of Cm (50mg/mL)
- Incubated overnight at 37deg in waterbath shaker

Made 4 aliquots of 50uL SE cells from original, some SE cells still left in original. All SE cells packaged in the box on the right.

Miniprep:

Other

Obtained UV Camera box for Discovery Day

Conclusion:

05.23.16 Victoria Day, BSB locked

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-24

TUESDAY, 5/24/16

Could not enter building, science post-poned

05.24.16 Miniprep 1-4I, 3-12K, 3-1B; Digestion those + pUC19, pIB184, mCherry

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-05-24

TUESDAY, 5/24/16

Entry By: Max

Purpose: Preparation for Discovery Day and ncessesary genes

Miniprep of 1-4I, 3-12K, 3-1B

Pipetted out 15mL of 20mL liquid -4 fridge overnights into 3 2mL eppendorfs

Then followed protocol

- For elution, all 150uL were eluted into the same 1.5mL

Digestion of 1-4I, 3-12K, 3-1B, pUC19, pIB184, mCherry

Single Digestions (pUC19, pIB184, mCherry)

135 uL rxn of 100uL DNA, 10uL SpeI HF, 10uL EcoRI-HF, 15uL cutsmart buffer

Double Digestions (1-4I, 3-12K, 3-1B)

122uL rxn of 100uL DNA, 10uL EcoRI-HF, 12uL cutsmart buffer

Incubated at 37C for 45 mins

Gel Electrophoresis

Added 2uL of 6x orange dye (Vivian's) to 10uL of DNA

05.27.16 Setting up overnight cultures for L. fermentum 793, L. brevis 765, and L. gasseri 33

Made with Benchling

Project: McMaster iGEM '16

Authors: Melodie Kim

Date: 2016-05-27

FRIDAY, 5/27/16

Entry by: Melodie Kim

Purpose: To grow overnight cultures for L. fermentum 793, L. brevis 765, and L. gasseri 33 (These three bacteria strains did not grow well in the previous overnight culture. L. brevis 884 was the only one that grew well. Tomorrow, we will be making glycerol stocks for all four strains.)

Reagents:
- MRS media

Procedures:

1. 3 mL of MRS Media was transferred to each snap on culture tubes (3 in total)
2. Inoculated the three bacteria cultures:
    a. Select a single colony from the bacteria plate using a sterile pipette
    b. add colony into the liquid MRS media by swirling the pipette tip inside the tube

05.25.16 Overnight cultures of Lactobacillus strains

Made with Benchling

Project: McMaster iGEM '16

Authors: Yosef Ellenbogen

Date: 2016-05-27

FRIDAY, 5/27/16

Entry by: Rayu Bhatt

Purpose: To prepare overnight cultures of *L. brevis 884, L. brevis 765*, *L. gasseri 33,* and *L. fermentum 793*.

Reagents:

- MRS Liquid Media
- *L. brevis 884, L. brevis 765, L. gasseri 33,* and *L. fermentum 793* plates

Protocol:

1. 4mL of MRS media was aliquoted into 4 overnight culture tubes with loose fitting caps
2. A small piece of the streak for strain was inocculated into one of the four culture tubes
3. The tubes were cultured overnight at 30C without shaking

Results:

- *L. brevis 884 yielded the highest, L. gasseri 33 and L. brevis 765 yielded small amount, and L. ferementum 793 did not work*.

05.28.16 Glycerol Stocks of L. gasseri 33, L. brevis 884, L. brevis 765

Made with Benchling

Project: McMaster iGEM '16

Authors: Yosef Ellenbogen

Date: 2016-05-28

SATURDAY, 5/28/16

Entry by: Yosef

Purpose: To prepare glycerol stocks of the lactobacilli obtained from the Surette lab

Reagents:

- Glycerol
- *L. brevis 884, L. brevis 765, L. gasseri 33 overnight cultures*

Protocol:

1. aliquotted 500ul of glycerol into 2ml tubes (used for glycerol stocks)
2. removed supernatant from the overnight cultures so that ~500uL of mrs media + pellet remained in the overnight tube
3. resuspended in the 500ul remaining and transfered into the glycerol tube so that it is a final dilution of 1:1 glycerol to MRS
4. placed the glycerol stocks in the -80 freezer for future use
5. **Note:** we did NOT make glycerol stock of L. ferementum 795 as the overnight cultures did not work. Additionally, the only culture that provided substantial growth was L. brevis 884 and it is possibe that the glycerol stocks for the L. brevis 765 and L. gasseri 33 do not have a lot of cell in them as compared to the L. brevis 884 stock.

06.01.16 Restocking Tips and general cleaning

Made with Benchling

Project: McMaster iGEM '16

Authors: Yosef Ellenbogen

Date: 2016-06-01

WEDNESDAY, 6/1/16

Entry by: Yosef, Rayu, Sam

Refilled 3 yelllow tip boxes and 2 white tip boxes however could not autoclave yet because it is out of order. Additionally we put the blue spreaders into new glass container to be autoclaved and cleaned the cuvettes. They were all put in the back room of the prep lab

DO NOT USE THESE ITEMS UNTIL WE CAN AUTOCLAVE THEM!

06.01.16 Transformation (pIB184-mCherry, Terminator, pIb184, pUC19); Digest (

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-01

WEDNESDAY, 6/1/16

Entry By: Max

Purpose: Complete reconstitution for all remaining Las and Lux parts, test to check if the DNA are proper through digest+gel, check UV spec issues

Transformation

1. pIB184-mCherry ligation (Ery.)
2. Reconstitution of 3-3F = double terminator (B0010+B0012) (Chl.)
3. pIB184 miniprep (Ery.)
4. pUC19 from Invitrogen (Amp.)

Followed Transformation protocol, modified as follows:

- 30min on ice for complexing (not 10min)
- 42sec at 42deg for heat shock (not 1min)
- 2min on ice for recovery (added)

Digest

1uL of PstI-HF
1uL of EcoRI-HF
1uL Cutsmart buffer (new, exp. 2018 version)
5uL DNA
2uL water

45min at 37deg

Gel

Check the digests above

[add image]

UV Spec

06.02.16 Liquid culture of 3-3F, Test of Ery plate

Made with Benchling

Project: McMaster iGEM '16

Authors: Yosef Ellenbogen

Date: 2016-06-02

THURSDAY, 6/2/16

Entry by: Yosef, Melodie

Liquid Culture

- 4mL LB lennox, 8uL of the Cm stock in freezer, with two colonies from the plate
- set overnight at 37 deg @150rpm

Erythromycin plate test

- plated 50ul untransformed S.E cells on erythromycin (double red lines) plate to test if erythromycin is working.
- if cells grow, then the plate is non-functional
- placed the plate in the autoclave room as it is warm there

Note: The result of this test will tell us if the transformation of pIB and pIB-mCherry on June 1 (see entry) worked. The results of those plates were lawns so it is likely the erythromycin isn't working but we will not know until we get the result of this plate test.

The two ery plates were placed with parafilm in the +4 fridge awaiting results of the plate test. THey will be made into a liquid culture if the S.E cells do not grow, and will be thrown out if they do grow.

06.03.16 Miniprep of 3-3F

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-03

FRIDAY, 6/3/16

Entry by: Tony

Miniprep - Standard protocol was used.
Product was labeled 3-3F miniprep and stored in -20

Ery plate neg. control resulted in lawn; it is concluded that the Ery plates did not have high enough conc. ery to select for pIB184.
Ery plates were discarded

06.07.16 Optimizing Erythromycin Conc.

Made with Benchling

Project: McMaster iGEM '16

Authors: Yosef Ellenbogen

Date: 2016-06-07

TUESDAY, 6/7/16

Entry by: Yosef

Purpose: To determine the proper concentration of erythromycing to be used in order to inhibit growth in bacteria not containing erythromycin resistance

Procedure:

- Stock of 7.34 mg/mL was made by Vivian and stored in the -20 freezer a few weeks back (see lab notebook May 10)
- based on literature, working concentration of erythromycin should be 300ug/mL in order to kill untransformed E. coli (1)
- Four liquid cultures were set up at 250, 300, 350 and 400 ug/mL erythromycin to determine the best culture conditions to inhibit growth (calculations below).
- 5mL LB Lennox and 10uL of S.E E. coli cells were added to the cultures with the respective antibiotic concentrations
- liquid culture tubes were placed in the water bathat 37C and 150rpm overnight.

Sample Calculation:

250ug/mL * 5mL = 1.25mg; 1.25mg/(7.34mg/mL) = 170uL

final concentration of 250ug/mL using a 7.34mg/mL stock requires 170uL ery stock in 5mL total volume.

References

(1) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4110107/

06.08.16 Erythromycin Plate Preparation

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-08

WEDNESDAY, 6/8/16

Entry By: Max

Purpose: Erythromycin Plate Preparation

Autoclave was fixed yesterday (Alastair).

Previous cultures

Plate preparation

LB Agar: 6.4g total = 3.2g in each bottle

Distilled H2O (special taps): 200mL total = 100mL in each bottle

Erythromycin (from stock): 6.8mL total = 3.4mL in each bottle - *approximately no erythromycin left*

Math:

32g LB Agar / 500mL DI water = 6.4g LB Agar / 200mL DI water

7340ug/mL * x = 250ug/mL * 200mL

x = 6.8mL of the stock into 200mL

06.10.16 pIB184 transformation

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-10

FRIDAY, 6/10/16

Entry By: Tony

Procedure

2uL pIB184 was transformed onto new Ery plates following standard protocol;
Plate was incubated in water bath at 37C

Note

Update: Transformation was successful

06.13/14.16 Overnight culture for pIB184 and miniprep of result

Made with Benchling

Project: McMaster iGEM '16

Authors: Dhanyasri Maddiboina

Date: 2016-06-15

WEDNESDAY, 6/15/16

Entry by: Yosef, Rayu

Purpose: Set up liquid culture of pIB184 in e.coli. Miniprepped the next day.

Protocol:

1. Picked a colony of *E.coli* containing pIB184
2. Inocculated in 5mL of LB Lenox liquid media with 300ug/mL (204uL of stock) of erythromycin
3. Incubated overnight in culture tube at 37 degrees, shaking at 200rpm
4. Culture was miniprepped the next day based on standard Thermo Scientific GeneJET Miniprep kit protocol
5. 50uL of DNA elution stored at -20 degrees

06.16.16 Stock solutions for L. brevis electrocomp

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-16

THURSDAY, 6/16/16

Entry By: Max

Procedure

Using materials from Dr. Surette's lab, made:

Concentrated Stock concentrations
NaH2PO4 = 200mM (1.2g in 50mL)
v = 50mL
mm = 119.98g/mol
m = 1.1998g = 1.2g
MgCl2 = 50mM (0.2380g in 50mL)
v = 50mL
mm = 95.211g/mol
m = 0.2380g
Sucrose = 2M (34.2296g in 50mL) —> solubility at room temp: 2g/mL = 0.006mol/mL = 6M
v = 50mL
mm = 342.2965g/mol
m = 34.2296g
Glycine = 20% (10g in 50mL)

Kim (2005)
Concentrated Wash (10x): 100mL
50mM NaH2PO4 —> Use 25mL of Stock
10mM MgCl2 —> Use 20mL of Stock
Add 55mL ddH2O

Large Volume of Stock Buffer (1x, use 5mL at a time): 50mL
1M Sucrose —> Use 25mL of Stock
3mM MgCl2 —> Use 3mL of Stock
Add 22mL ddH2O

If the above protocol fails, use Posno (1991).

Note

These materials are held in Dr. Surette's lab, on Michelle's desk, labelled iGEM.
MRS has been ordered, should arrive tomorrow.
We need to autoclave bottles to bring ourselves OR give them to Michelle in Dr. Surette's lab on Monday before 3pm to be autoclaved Tuesday morning.
Create Wash and Buffer during the Growth step.

06.21.16 Making MRS liquid media

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-06-22

WEDNESDAY, 6/22

Entry By: Rayu, Tony, Max

Purpose: creating 500mL of MRS liquid medium for *L.Brevis 884*

Reagents:

- 475mL MilliQ water
- 27.5g MRS powder
- 25mL 10% Glycine stock solution

Protocol:

1. Added 27.5g of MRS powder to 475mL of non-sterile MilliQ water
2. Heated solution to near-boil with constant stirring to ensure dissolution of all solid particles
    a. Note: The colour of the MRS became a deep amber colour at this point
3. Autoclaved the MRS at 121 degrees for 20 minutes
4. Allowed to cool added 25mL of 20% glycine stock to create a final 1% glycine MRS solution
5. Stored at 4 degrees until needed

06.23.16 Making Electrocomp L.brevis

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-06-23

THURSDAY, 6/23

Entry By: Rayu, Tony, Max

Purpose: Create electrocompetent L.brevis aliquots for future use

Reagents:
L.brevis 884 overnights
Wash Buffer (50mM NaH_2PO_4, 10mM $MgCl_2$), on ice
Electroporation Buffer (1M Sucrose, 3mM $MgCl_2$), on ice
MRS media (1% glycine added)
Dry Ice

Procedure

1. 400mL MRS media was inoculated with 8mL L.brevis overnight culture and grown at 30C with 75rpm shaking until OD600 = 0.2 (~2.5h)
2. A sample of the culture was gram stained
    a. The fixation step was inadvertently skipped so few cells remained on slide
    b. No gram negatives were found; some gram positives were found.
    c. Results indicated for continuation of procedure
3. Culture was split into 10 aliquots of 40mL and cooled on ice for 10 mins
4. Aliquots were centrifuged at 4C at 2900xg for 5 mins
5. Supernatant was discarded and pellets were resuspended in 40mL each of wash buffer by vortex
    a. Cells were centrifuged to pellet as before
6. Supernatant discarded; resuspension in 20mL each of wash buffer
    a. Cells were centrifuged to pellet as before
7. Supernatant discarded; resuspension in 10mL each of wash buffer
8. Aliquots were combined into 2 tubes of 50mL each
    a. Cells were centrifuged to pellet as before
9. Supernatant discarded; resuspension in 2mL each of electroporation buffer
10. 40 aliquots of 100uL were made into prechilled cryotubes and flash frozen on dry ice
11. Cells were stored at -80C in BSB freezer

Verification of tests and contamination

Gram Staining: To check for Gram-negative bacteria in the sample before washed

Procedure: Followed standard protocol, in fume hood in Dr. Surette's lab. Help from Fiona (?).

Note: Do not mix crystal violet or chemical waste with non-chemical waste, as it has to be disposed of in special jugs if the bleaching fails, and the jugs are expensive, so aim to reduce the amount of fluids in chemical waste.

Error: Forgot to heat stick the bacteria to the microscope slide; luckily, some bacteria did stay.

Results: Only pink (Gram-positive = L. brevis) cells present

MacConkey (Mac) Plate: To check for Gram-negative bacteria growth

Procedure: Added growth solution to Mac Plate; used several (aim for use of 5) glass beads as spreaders; dried, then dumped glass beads in a special jar from Michelle for waste glass beads (contained ethanol and other beads); flipped and marked the plate, and placed in the top left incubator on the middle rack in Dr. Surette's lab.

06.24.16 Checking Mac Plate

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-06-24

FRIDAY, 6/24

Entry by Rayu Bhatt

Results:

- Mac Plate selective from gram negative bacteria streaked with *L. Brevis 884* stock used to make electrocomp cells yielded no colonies, supporting the electrocomp cells as being *L. Brevis*.

06.28.16 Making MRS Agar Plates

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-06-28

TUESDAY, 6/28

Entry by: Yosef, Rayu

- dissolved 70g of MRS Agar in 1L of dH20
- heated up for 15minutes on medium heat and mixed with stir bar until homogenous
- autoclaved on cycle 1 (~15 min)

note: the autcolave caused about half of the mrs agar to spill over which that was cleaned up promptly.

- the remaining 400ml was set to cool and 300ug/ml (0.12g) of erythromycin powder was added and mixed using a stir bar
- MRS Agar was poured into one sleeve of plates (minus three plates) and set in 4deg fridge
- plates were marked with two red lines indicating erythromycin and one green line indicating MRS

Note: there is no erythromycin left - there was only enough to fulfill the 400mL remaining after the spill which means if none had spilled, we would still have only been able to use 400ml of it.

Note: the protocol used was different than for Agar plates and it was followed according to instructions on the MRS Agar Powder

06.29.16 Electroporation of L. Brevis w/ pIB184 and pIB184+mCherry

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-07-01

FRIDAY, 7/1

Entry By: Max, Tony

Purpose: Electroporation of L. Brevis w/ pIB184 and pIB184+mCherry

Reagents:
L.brevis 884 aliquots (2)
pIB184 DNA
pIB184+mCherry DNA
Electroporation Buffer (1M Sucrose, 3mM MgCl2), on ice
MRS media (1% glycine added)
Dry Ice

Procedure
Use nanodrop to find concentration of DNA
- Using the appropriate buffer (Elution buffer for pIB184 and ligation buffer (diluted from 10x) for pIB184+mCherry)
- pIB184 = 150 ng/uL
- pIB184+mCherry = 300 ng/uL

Add 2µL (25 ng/µL) of plasmid DNA to 100 ul electrocomp cell aliquot of ice-cold cell suspension (75ng/uL was used for pIB+mCherry due to unclean reaction)
Electroporate at 12.5 kV/cm (pulse number = 10, puse interval = 500 ms)
- The electroporation cuvette had a width of 0.2cm; therefore, setting at 2.5kV/cm
- No sparking
- Actual result: pIB184+mCherry: 4ms at 2370V
  pIB184: 4.5ms at 2370

Dilute electroporated cells to 1ml in MRS broth and incubate at 37°CC for 3 hours (water bath)
- Incubated for 2hrs inside the electroporation cuvette before being transferred into an eppendorf
  - Incubated under anaerobic conditions

Plate bacteria onto MRS agar plates with appropriate antibiotic (the Erythromycin plates made yesterday)
- This was let grow overnight at room temperature

An overnight was also prepared (without antibiotics)

Note: the procedure on http://openwetware.org/wiki/Electro-transformation_of_Lactobacillus_spp. for Kim (2005) is wrong, use that found on http://openwetware.org/wiki/Lactobacillus_transformation_%28Kim_2005%29 or http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2005.02563.x/abstract;jsessionid=AE53AE8283F0EE6BBF5CA53167A6C943.f04t02

07.06.16 Checking plates, checking liquid cultures, miniprep, gel electrophoresis

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-06

WEDNESDAY, 7/6

Entry by: Rayu Bhatt, Maxwell Ng

Observations from previous plates of electroporated *L.Brevis 884*:

- pIB184 and pIB184-mCherry mac plate: No growth
- pIB184 and pIB184-mCherry MRS Agar plate (10ug/mL Erythromycin): No growth
- Electroporation control MRS Agar plate (10ug/mL Erythromycin): No growth

Observations from liquid cultures of electroporated *L.Brevis 884* (Table 1):

- Note that *new cultures* are those of July 5th, 2016. Consult Yosef Ellenbogen for details.

Table1

	A	B	C
1	**pIB184 from previously picked colony (10ug/mL Ery)**	**pIB184-mCherry from previously picked colony (10ug/mL Ery)**	
2	Growth	Growth	
3	**pIB184 new culture (10ug/mL Ery)**	**pIB184 new culture (5ug/mL Ery)**	**pIB184 new culture (1ug/mL Ery)**
4	No growth	No growth	No growth
5	**pIB184-mCherry new culture (10ug/mL Ery)**	**pIB184-mCherry new culture (5ug/mL Ery)**	**pIB184-mCherry new culture (1ug/mL Ery)**
6	No growth	No growth	No growth

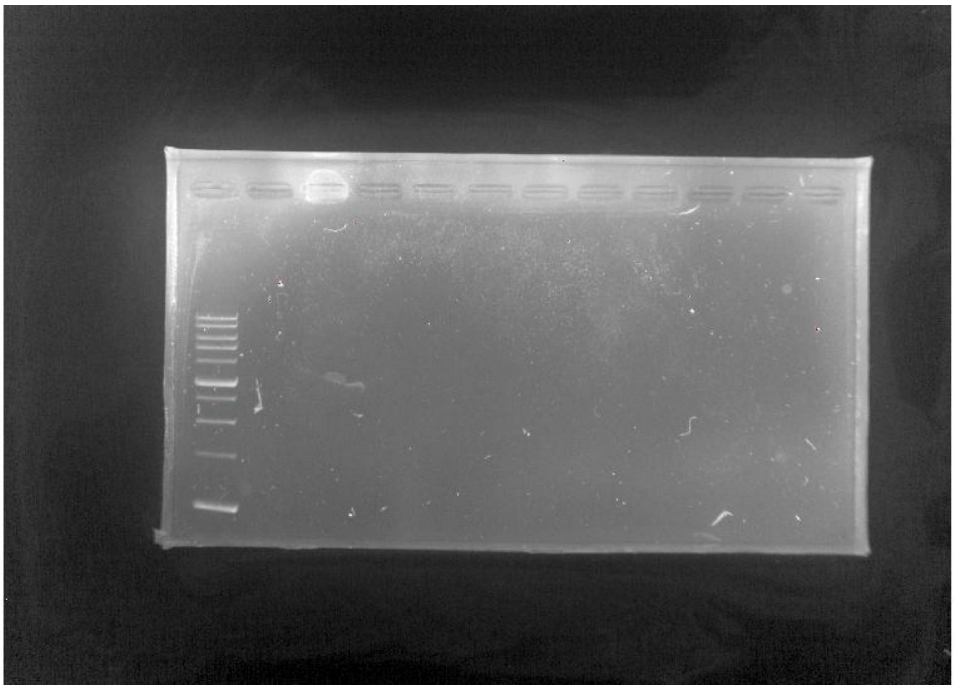
The two liquid cultures with growth (Table 1, 1A and 1B) were miniprepped and DNA was stored at -20 celcius until it can be run on a 1% agarose gel to confirm successful transformation.

The miniprep DNA (pIB184 and pIB184-mCherry) were digested (EcoRI-HF and SpeI-HF) and use run on the following gel:
1-ladder
3-pIB184
5-pIB184-mCherry

Errata Note: The solution solidified too quickly (it seems that the room was colder than usual), not allowing red safe to combine; microwaved again, and added 2.0uL RedSafe again

Analysis: Contamination likely of pIB184; otherwise no DNA in anything

Maxwell Ng 2016-07-06 17hr 37min.jpg



07.11.16 Making Ery Plates, running Gel with original pIB and E.coli pIB minipreps used for electroporation

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-12

TUESDAY, 7/12

Entry By: Tony, Max, Rayu

Reagents for gel electrophoresis:

- 50mL 1% Agarose Gel
- 2.5uL 20000x Red Safe
- TAE Buffer
- *E.coli* miniprep of pIB184 plasmid (now used for electroporation of *L. Brevis)*
- Original pIB184 obtained from Mahony Lab

Reagents for MRS agar plates:

- MRS Agar
- Erythromycin in Ethanol

Results of Gel Electrophoresis:

- Gel electrophoresis results of 10kb ladder (lane 1), original pIB (lane 3), miniprepped pIB (lane 5)
- Plasmid is safely confirmed and can continue to be used in electroporation

MRS agar plates preparation

MRS agar plates of different erythromycin concentrations made.
Concentrations of Ery:

Two possibly contaminated.

Addition of electrocompetent cells

Two 100uL aliquots of electrocompetent *L. brevis* cells were recovered (no electroporation) into 5mL of MRS broth. After 3hr incubated at 30C, it was concentrated (centrifugation and removal of supernatant) and then plate onto the 5 different Ery concentrations (one plate each). All plates incubated at 33C for overnight.

One of the contaminated plates (specifically, __) had aliquot cells added, the other (___) did not. The other was added to the incubator as well as the rest of the plates to test if it had any contamination that will grow, as a control for the other contaminated plate.

07.12.16 pIB184 Electroporation, PCR

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-07-12

TUESDAY, 7/12

Entry By: Tony

PCR

Reagents

Lyophilised DNA: DARPIN-Cell Wall Anchor (cwa), Las gBlock 1 (Las1), Las gBlock 2(Las2)
Oligonucleotide Primers: Common Forward (FP), Common Reverse (RP), Las Stitch (SP)
ddH2O
Platinum PCR Supermix

Procedure

Lyophilised DNA was resuspended according to instructions on package
gBlocks -> 10ng/uL
Primers -> 100mM

Aliquots were taken and stock dilutions were made using ddH2O:
gBlocks -> 1ng/uL
Primers -> 5mM

All were stored in -20C freezer

5x 50uL PCR reaction mixtures were made for each DNA:
45uL Platinum PCR Supermix
1ng DNA (1uL * 1ng/uL DNA)
0.25mM each Corresponding Forward and Reverse Primers (2.5uL * 5mM DNA)

Primers Used

	A	B	C	D
1	**DNA**	cwa	Las1	Las2
2	**Forward Primer**	FP	FP	SP
3	**Reverse Primer**	RP	SP*	RP

*Note: It was later found that the SP was shipped as ssDNA and contains no complementary strand; it is therefore unsuitable for use as a **reverse** primer

The reactions were cycled using the following settings:
94C * 2m - Initial Melt
12 Cycles:
94C* 30s - Melt
55-69C * 30s - Anneal
68C * 2m - Elongate
68C * 5min - Final Elongation
4C - Hold

07.13.16 Gel Electrophoresis of PCR amplicons, Stitch PCR attempt 1, Las1 PCR attempt 2

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-07-15

FRIDAY, 7/15

Entry by: Rayu Bhatt, Tony Chen

Reagents:

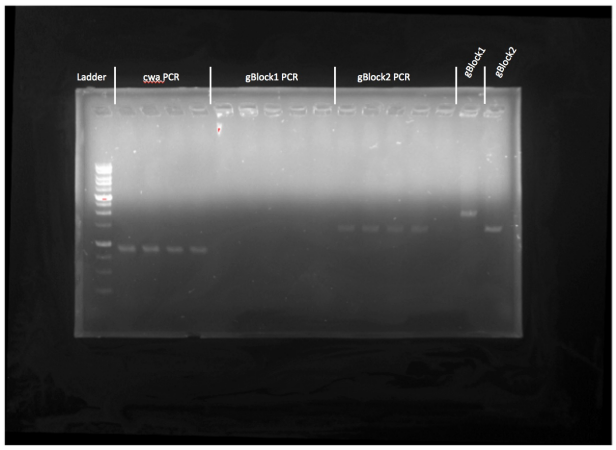
- PCR Amplicons of gblock1, gblock2 and cwa-anti-Her2 DARPIN
- Reagents needed in protocol for 1% agarose gel electrophoresis

Protocol:

- Lowest annealing temp for cwa PCR was excluded from the gel
- 10uL of each PCR reactions was mixed with 2uL of loading dye
- Each was run on a gel at 100V for 30 mins

Results:

Screen Shot 2016-07-15 at 1.27.47 PM.png



PCR

Reagents

Las1 Amplicon

Las2 gBlock

Primer Stocks: Common Forward (FP), Common Reverse (RP)

Platinum PCR Supermix

Procedure

4x50uL PCR reaction mixtures were made each:

Stitch

45uL Platinum PCR Supermix

1uL Las1 Amplicon Incorrect Amplicon Used

1ng Las2 gBlock (1uL * 1ng/uL DNA)

0.25mM each FP, RP (2.5uL * 5mM DNA)

Las1
45uL Platinum PCR Supermix
1ng Las1 gBlock (1uL * 1ng/uL DNA)
0.25mM each FP, SP (2.5uL * 5mM DNA)

A beaker of water was microwaved to 100C and allowed to cool to 94C
The reactions were Incubated in the water, and allow to cool to 50C for annealing
The reactions were aliquoted into 5x50uL reactions

The reactions were cycled using the following settings:
69C * 3m - Initial Elongation
12 Cycles:
    94C* 30s - Melt
    55-69C * 30s - Anneal
    69C * 3m - Elongate
69C * 5min - Final Elongation
4C - Hold

07.15.16 Stitch PCR attempt 2, pIB184 Liquid Cultures, PCR Cleanup of Las gBlock2, gel preparation

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-16

SATURDAY, 7/16

Entry by: Maxwell Ng, Tony Chen, Melodie Kim

PCR

The procedure was identical to that used in stitch attempt 1; with the alteration that the Las1 gBlock was used in place of the Las1 amplicon, and the Las2 amplicon was used in place of the Las2 gBlock:

Procedure

a 50uL PCR reaction mixtures was made:
45uL Platinum PCR Supermix
1ng Las1 gBlock (1uL * 1ng/uL DNA)
1uL Las2 Amplicon
0.25mM each FP, RP (2.5uL * 5mM DNA)

A beaker of water was microwaved to 100C and allowed to cool to 94C
The reaction was Incubated in the water, and allow to cool to 50C for annealing

The reactions were cycled using the following settings:
69C * 3m - Initial Elongation
12 Cycles:
    94C * 30s - Melt
    58C * 30s - Anneal
    69C * 3m - Elongate
69C * 5min - Final Elongation
4C - Hold

PCR Cleanup if Las gBlock2

A gel was prepared to run the results of the Stitch attempt (ran on Sunday by Melodie)

PCR DNA cleanup was performed (Thermofischer GeneJet kit, protocol in kit) on 2 aliquots of Las.Amplicon2 PCR products.

pIB184 Liquid Cultures from plate

07.14.16 Gel electrophoresis of Stitch PCR

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-07-17

SUNDAY, 7/17

Entry By: Tony

Results

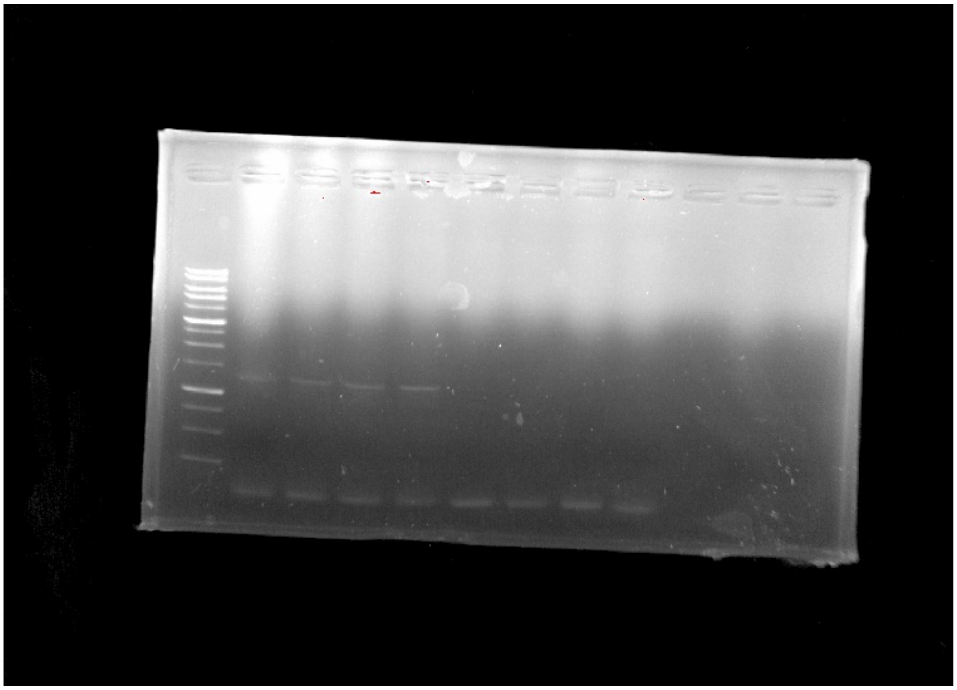
Lanes 2-5: Stitch, Lanes 6-9: Las1, Lane 11: Las1 gBlock, Lane 12: Las2 gBlock

Las 1 gBlock failed to amplify - *SP did not function as a reverse primer because it lacked a reverse strand

Stitch PCR failed and instead amplified Las2 - *incorrect gBlock/amplicon was used.

gBlock control lanes failed to show signal - *possibly loading error

Maxwell Ng 2016-07-14 13hr 36min.png



07.19.16 Electroporation, Ligation of Las parts, Gel Electrophoresis of Ligation, Stitch PCR attempt 3

Made with Benchling
Project: McMaster iGEM '16
Authors: Maxwell Ng
Date: 2016-07-19

TUESDAY, 7/19

Entry By: Yosef, Rayu

Electroporation

- Electrocomp *L. Brevis* electroporated (2 aliquots as control, 2 aliquots with pIB184)
- One control aliquot and one pIB184 transformation was plated each on a separate 8ng/uL Erythromycin MRS agar plate
- NOTE: Cross contamination of control and pIB184 electroporated cells was accidentally plated on a single 14ng/uL Erythromycin MRS agar plate (ask Rayu for details).
    - This can only be used to indicate if cells survived electroporation, but this is not indicative of selection for control or pIB184 transformed cells.
    - If control does not grow at 8ng/uL Erythromycin but pIB184 transformation does, then it is likely that any growth on the cross contaminated plate could be pIB184 transformed *L. Brevis.*
- Plates stored at 37 degrees in Dr. Surette's lab.

- two 14ng/uL (erythromycin) MRS agar plates remain unused for future use in 4 degree fridge
- three 8ng/uL (erythromycin) MRS agar plates remain unused for future use in 4 degree fridge

Ligation of Las parts

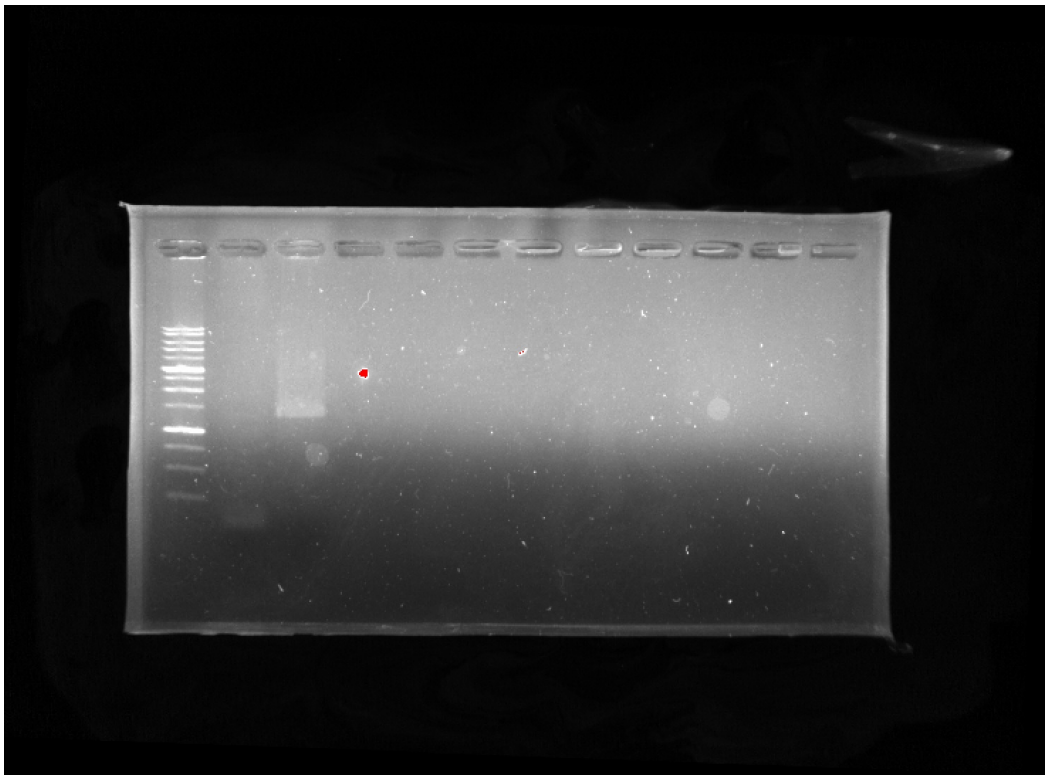
Tony pls

Gel electrophoresis of Las ligation and second attempt of stitch PCR gel

- 1% agarose gel prepared
- GeneDireX 10kb ladder run in lane 1
- Previously attempted stitch PCR amlicon run in lane 2
- New Las ligation run in lane 3 (Please note that remainder of ligation is at -20 and must be run and gel extracted as well)

Resutls: Stitch PCR shows undersized amplicon. Ligation looks successful and needs to be gel extracted, and remainder must be run and gel extracted as well (see note above).

Screen Shot 2016-07-19 at 6.55.56 PM.png



- Weight of gel fragment cut from gel is ~0.620g (please see exact weight written on side of eppendorf, and use approprate amount of buffer according to 1:1 ratio discussed in iGEM Gel Extraction protocol!

Stitch PCR

- diluted down the stock las gblocks. 2ul in 38ul elution buffer to final concentration of 0.5ng/ul

volumes in PCR tube (total volume 54ul):
- 2.5ul forward primer (from the 5mM diluted stock - black writing on eppendorf)
- 2.5 ul reverse primer (from the 5mM diluted stock - black writing on eppendorf)
- 2ul las gblock 1 (1ng)
- 2ul las gblock 2 (1ng)
- 45ul Master Mix

- heated 100ml beaker of water to boil in microwave. placed thermometor in it and the pcr tube using a tweezer. Held in beaker until the temperature decreased to 50 and then placed in thermocycler.

Themocycler setting:

3:00 (initial elongation) @ 72C

12 cycles:
0:30 (Denaturation) @ 94C
0:30 (Anneal) @ 58C
3:00 (Elongation) 72C

5:00 (Final Elongation) @72C

Hold @ 4C

Storage

-The 0.5ng/ul is the stock that should be used for subsequent PCRs' and there is 38uL left from each gblock. They were placed in the primer rack and appropriately labelled.

Contact Yosef for any questions about placement or details of this procedure

07.20.16 Gel extraction of Stitch PCR attempt 2, Gel electrophoresis of Stitch PCR attempt 3

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-20

WEDNESDAY, 7/20

Entry by: Max, Fei Fei

Overnight pellet

Centrifuged and pelleted, kept at -20C.

Gel extraction of Stitch PCR attempt 2

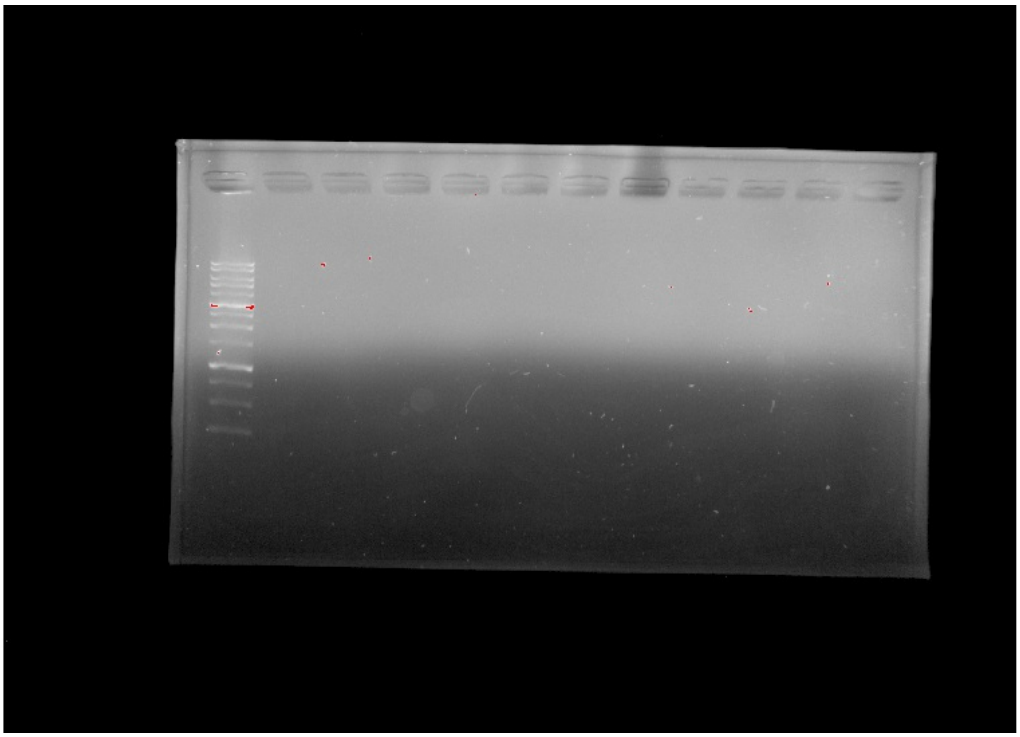
Followed protocol, did additional optional step for sequencing purposes (as outlined in the GeneJet protocol).

Gel electrophoresis of Stitch PCR attempt 3

20uL DNA + 2uL 6x loading dye (orange)

Lane 1: GeneDireX 1kb Ladder
Lane 3: residual, some was spilled due to use of larger pipette
Lane 5: Stitch attempt 3 DNA (~10uL)

Maxwell Ng 2016-07-20 15hr 21min.png



07.25.16 Lysozyme assisted miniprep of pIB184 from old cell pellet, gel electrophoresis of minipreps and of stitch PCR trail #_ (Entry incomplete)

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-07-25

MONDAY, 7/25

Entry by: Rayu Bhatt, Tony Chen

Purpose: To try out two differing methods of lysozyme assisted minipreps on a supposed *L. Brevis 884* cell pellet containing pIB184. To evaluate validy of stitch PCR trial __.

Reagents:

1. 100mg/mL lysozyme solution (in -20 box with yellow sticker on lid)
2. Tris
3. HCl
4. Na_2EDTA
5. Sucrose
6. GeneJET Miniprep kit (excluding RNase A resuspension buffer)

Method 1: This method was proposed by a paper discussing the isolation of plasmids from lactobacillus bacteria[1].

Protocol: Preparation of 10x stock solution of 500mM Tris-HCl, 50mM Na_2EDTA, pH 7.5

1. 1.211g of Tris and 0.372g of Na_2EDTA were dissolved in a minimum measured volume of nanopure water
2. concentrated HCl was gradually added (and measured) until a pH meter read a pH of 7.5
3. Water was then added again to achieve a final volume of 20mL based on the amount of water and HCl added previously
4. A 1X stock of this was used with a 1mg/mL working concentration of lysozyme to a 1mL final volume. This was added to a bacterial pellep that had already been isolated. The mixture is set on ice for 1 hour.
5. Lysis solution was then added based on the GeneJET Miniprep kit protocol
6. Kit protocol was followed to completion, excluding resuspension using RNase A resuspension buffer.

Discussion: The results of this method were not as promising as that of method 2, as addition of lysis buffer from the GeneJET miniprep kit did not reduce the transleucency of the cell suspension. The solution remained cloudy after the addition of lysis buffer, which is a telltale sign of inefficient cell lysis. This is potentially due to the low final concentration of lysozyme in solution upon the initial incubation step. These results may not be conclusive as the bactrial cell pellet used was very old, and potentially was not a true culture of *L. Brevis 884*.

Method 2: This method was proposed on a research gate thread[2].

Protocol: Tony pls

1. pls

References:

1. http://link.springer.com/article/10.1007/BF01576043
2.

07.26.16 pIB184 Electroporation, Stitch PCR attempt 5s

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-26

TUESDAY, 7/26

New PCR Protocol: 2 reactions in parallel (normal temp & low temp)

1. Add 45uL of Platinum PCR supermix
2. Add 0.5ng = 1uL Las gBlock 1 from eppendorf (0.5ng/uL)
3. Add 0.5ng = 1uL Las gBlock 2 from eppendorf (0.5ng/uL)
4. *Program the thermocycler to the procedure below:*
    a. Volume = 47uL
    b. 3:00 @ 94C (Hot Start and dsDNA separation)
    c. 15 cycles:
        I. 0:30 (Denaturation) @ 94C
        II. 0:30 (Anneal) @ 58C & 33C (machine requires temps to be within 25C of one another)
        III. 3:00 (Elongation) @ 72C
    d. FINAL: 5:00 (Elongation) @ 72C
    e. Hold 4C
5. Add 15uL of Platinum PCR supermix
6. Add 2.5uL of forward primer and 2.5uL of reverse primer (diluted eppendorfs)
    a. Volume = 67uL
    b. 3:00 @ 94C (Hot Start and dsDNA separation)
    c. 15 cycles:
        I. 0:30 (Denaturation) @ 94C
        II. 0:30 (Anneal) @ 58C & 33C
        III. 3:00 (Elongation) @ 69C
    d. FINAL: 5:00 (Elongation) @ 69C
    e. Hold 4C

Two labels: S5 58 and S5 33 (for temperatures).

07.28.16 CWA-DARPin and pIB184 Extraction, PCR of Las1, Lux1, Lux2, cwa2

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-07-28

THURSDAY, 7/28

Entry by: Samaher, Dhanyasri, Max, Melodie

Purpose: To prepare overnight cultures of PIB electrophoresis plates and to extract gel slices from (the gel that Rayu ran)

Reagents for Overnight Cultures:

1. 4.91 mL MRS (x2)
2. 87.5 uL Erythromycin (x2)

Protocol for Overnight Cultures:

1. Obtain two 5 mL tubes
2. Pipet 4.9125 mL of MRS and 87.5 uL Erythromycin into each tube
3. Enoculate the media in the first tube with a colonie from the 14 ng/uL plate
4. Enoculate the media in the second tube with a colonie from another 14 ng/uL plates

Observations for Overnight Cultures:

1. Zero colonies observed for both 8 ng/uL plates
2. One colonie observed in first 14 ng/uL plate and three colonies observed in second 14 ng/uL plate

1: Ladder
2: Stitch attempt 5, 33 degree
3: Melodie's PCR
4: Stitch attempt 5, 58 degree
5: Las 2 PCR cleanup
6: Stitch attempt 3 (Tony's "I told you so")
7: Digestion of PCR CWA-DARPIN
8: Digestion of original CWA-DARPIN

Reagents for Gel Extraction:

1. Refer to GeneJET Gel Extraction Kit

Protocol for Gel Extraction:

1. Cut out gel slices from lanes 2, 3, 4, 7, 8 at 3.2 kb for lanes 2-3 and at 1000 bp for lanes 7-8
2. Weighed each gel pieces

Gel piece weights:
Lane 2: 205 mg
Lane 3: 147 mg
Lane 4: 187 mg
Lane 7: 106 mg
Lane 8: 144 mg
3. Followed protocol as stated in GeneJET Gel Extraction Kit

PCR

```
Lux gBlock 1 will use:
Fwd: FP
Rev: Lux Stitch Reverse (R1)

Lux gBlock 2 will use:
Fwd: Lux Forward Stitch (F2)
Rev: RP

Lux gBlock 1 will use:
Fwd: FP
Rev: Lux Stitch Reverse (R1)

Lux gBlock 2 will use:
Fwd: Lux Forward Stitch (F2)
Rev: RP
```

Stock: (buffer)
Primers: 100uM
DNA: 10ng/uL

Working aliquot: (water)
Primers: 5uM
DNA: 0.5ng/uL

Made doubles of each 52uL reaction. One is made using Platinum Supermix, one made using Platinum Supermix that was left to defrost for 1.5h (marked X). PCR tubes marked respectively.

2.5uL of each primer
2uL of template DNA

PCR Protocol:
3:00 @ 94C (restarted before 3:00 over to switch to 14X cycles)
CYCLE: 14X
    0:30 @ 94C
    0:30 @ 60C
    2:00 @ 69C
5:00 @ 69C
inf @ 4C

07.29.16 L. Brevis lysozyme assisted miniprep, Gel (Las1, Lux1, Lux2, cwa2), overnight of DH5a

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-01

MONDAY, 8/1

Entry by: Rayu Bhatt

Purpose: To attempt lysozyme assisted miniprep of *L.brevis* 884 electroporated with pIB184 to isolate any plasmids for verification later. To run a gel of Las1, Las1x, Lux1, Lux1x, Lux2, Lux2x, cwa2, cwa2x PCR attempts to verify successful PCR. Overnight culture of DH5a transformed with pIB184-Km made to verify kanamycin resistance and successful transformation.

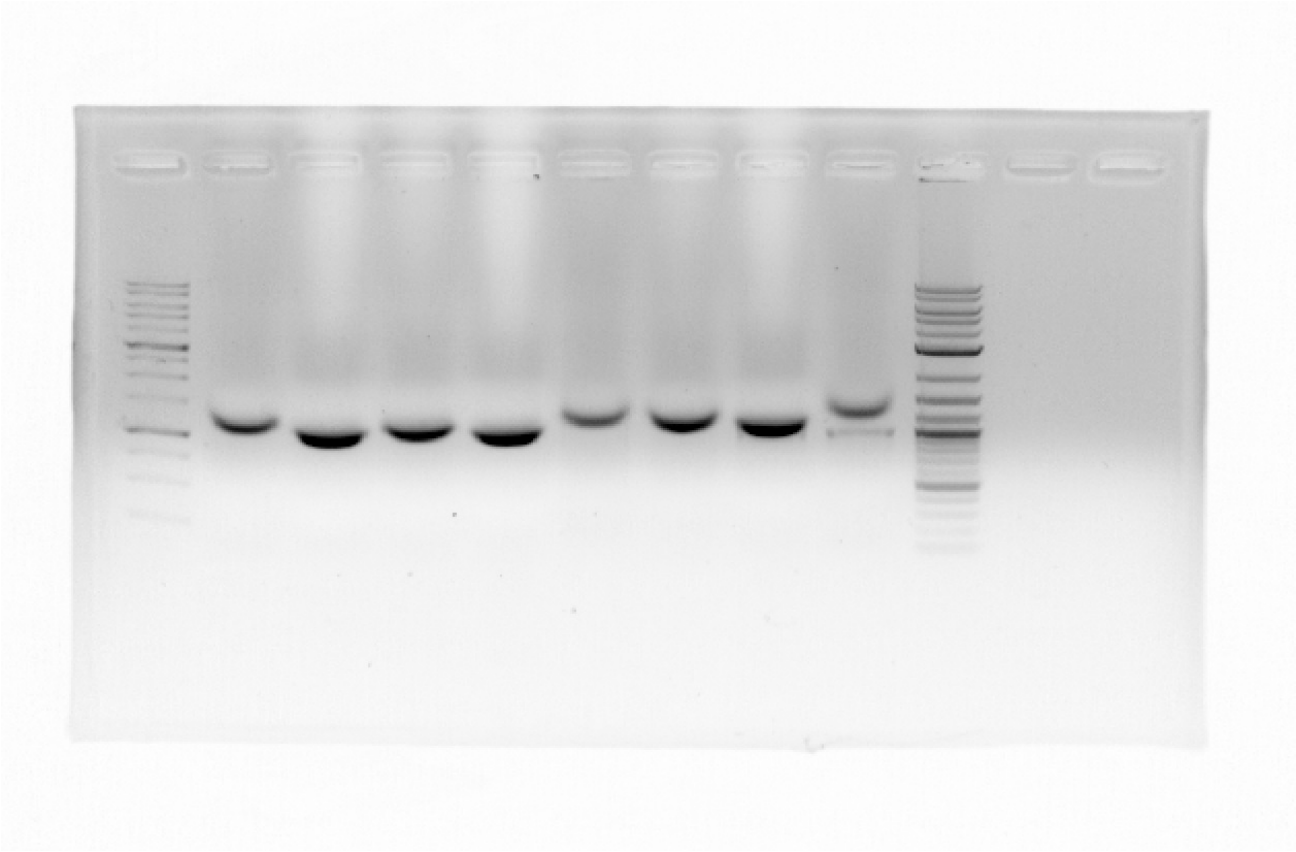
Miniprep:

Gel:
Previous PCR attempts were run on a 1% agarose gel with a 1kb GeneDireX ladder (lane 1) and a 2log ladder (lane 10)
10uL of all PCR amplicons were loaded with 2uL of 6X loading dye.
5uL of the 1kb ladder was loaded.
6uL of the 2 log ladder was loaded.

Lane order:

1. 1kb ladder
2. las1 = 1891 b
3. las1x = 1891 bp
4. lux1 = 719 bp
5. lux1x = 719 bp
6. lux2 = 1379 bp
7. lux2x = 1379 bp
8. cwa2 = 1037 bp
9. cwa2x = 1037 bp
10. 2 log ladder

Screen Shot 2016-07-29 at 6.08.10 PM.png



Overnight culture:
5mL of a 0.0114mg/mL kanamycin LB Lennox liquid medium was used to inocculate a colony of pIB184-km transformed *E.coli* DH5a.

08.02.16 Gel electrophoresis of diluted PCR products & pIB184 L. brevis minipreps

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-02

TUESDAY, 8/2

Entry by: Maxwell Ng

Purpose: Verify if electroporation and miniprep of L. brevis with pIB184 was finally successful; attempt the previous gel of PCR products by dilution (Alison says there are some salt effects and that it is over-saturated wells, may be causing some of the effects)

Gel electrophoresis

6uL for each ladder (as in previous)

Non-x PCR product (1/5 previous): 2uL DNA + 2uL 6x orange ladder + 8uL nuclease-free water (from Dr. Surette's lab)

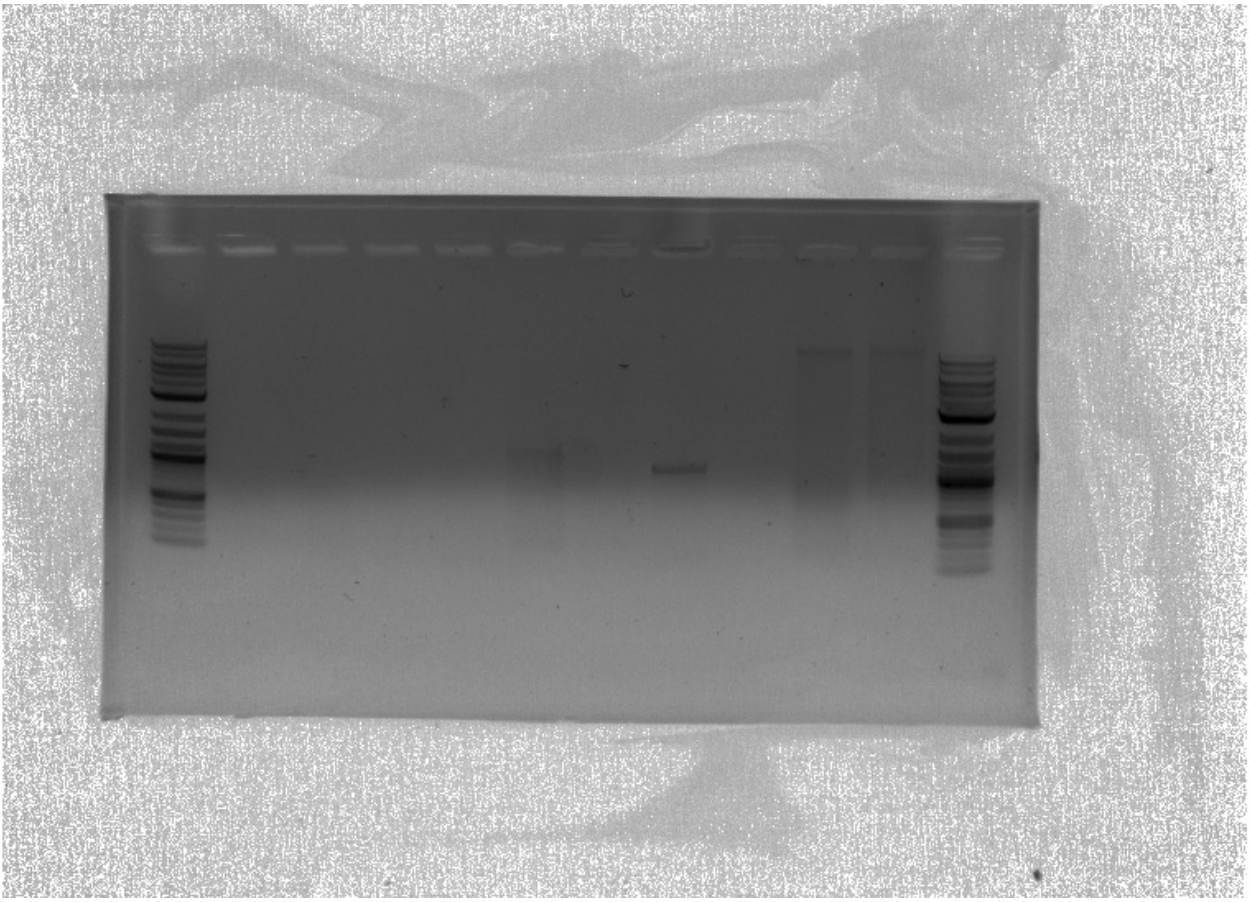
x PCR product (1/10 previous): 1uL DNA + 2uL 6x orange ladder + 9uL nuclease-free water (from Dr. Surette's lab)

2 minipreps were present - A & B noted to distinguish

Lane order: (superimposable on previous gel by Rayu Bhatt)

1. 2-log ladder
2. las1 = 1891 b
3. las1x = 1891 bp
4. lux1 = 719 bp
5. lux1x = 719 bp
6. lux2 = 1379 bp
7. lux2x = 1379 bp
8. cwa2 = 1037 bp
9. cwa2x = 1037 bp
10. pIB184 miniprep A
11. pIB184 miniprep B
12. 2-log ladder

Maxwell Ng 2016-08-02 17hr 11min.png



Note: no more 6x orange ladder

08.03.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-04

THURSDAY, 8/4

08.04.16 PCR of LasStitch9, MelStitches, and LuxStitch1

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-04

THURSDAY, 8/4

Entry by: Maxwell Ng

Purpose: Re-attempt PCR of the Las system, and new attempt of Stitch of Lux system.

PCR

Ran PCR on stitch for both Lux and Las. Lux was diluted to 0.1ng (as shown below). As a multiple temperatures were used, Lux and Las were doubled in their reaction: Lux 69, Lux 66, Las 69, and Las 66 are the labelled tubes.

In only the second phase, both of Melodie's Stitch PCR gel extractions were ran, one had a label on the 1.5mL eppendorf, and one did not. The PCR tubes were labelled as M and ML, respectively. 4uL of Melodie's DNA was ran in each PCR tube. Was there primers though?

These new protocols are based on several different papers amalgamated together.

New New PCR Protocol: 2 reactions in parallel (normal temp & low temp) Lux

1. Add 45uL of Platinum PCR supermix
2. Add template DNA
    a. For Lux: Add 0.1ng = 2uL Las gBlock 1 from eppendorf (0.5ng/uL), Add 0.1ng = 2uL Las gBlock 2 from eppendorf (0.5ng/uL)
    b. For Las: Add 1ng = 2uL Las gBlock 1 from eppendorf (0.5ng/uL), Add 1ng = 2uL Las gBlock 2 from eppendorf (0.5ng/uL)
3. *Program the thermocycler to the procedure below:*
    a. Volume = 48uL
    b. 3:00 @ 94C (Hot Start and dsDNA separation)
    c. 4X cycles (5 total):
        I. 0:30 (Denaturation) @ 94C
        II. 0:30 (Anneal) @ 60C (machine requires temps to be within 25C of one another)
        III. 3:00 (Elongation) @ 69C
    d. FINAL: 5:00 (Elongation) @ 69C
    e. Hold 4C
4. Add 2.5uL of forward primer and 2.5uL of reverse primer (diluted eppendorfs)
    a. Volume = 53uL
    b. 3:00 @ 94C (Hot Start and dsDNA separation)
    c. 14X cycles (15 total):
        I. 0:30 (Denaturation) @ 94C
        II. 0:30 (Anneal) @ 66C & 69C
        III. 4:00 (Elongation) @ 69C
    d. FINAL: 5:00 (Elongation) @ 69C
    e. Hold 4C

Note: Stitch = Stitch Overlap Extension = SOE PCR

08.09.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-08-09

TUESDAY, 8/9

A pET26b transformation was plated onto Kanamycin LB, but was unsuccessful

Autoclave cycle together, shortest time on Liquid

Kanamycin agar plates

Concentration: as old, (0.040mL*100mg/mL)/350mL = 0.0114mg/mL

Number made: 8 x 20mL = 160mL

Mark: 2 black lines

Stock Kan: 100mg/mL

C V = c v

100mg/mL * X = 0.0114mg/mL * 100mL

Using a 125mL flask: add 0.0114mL (11.4uL of Stock Kan) into 100mL autoclaved

Erythromycin MRS plates

Concentration: 0.01ug/mL, 0.1ug/mL, 0.50ug/mL, 1.0ug/mL, 2.0ug/mL

Number made: 1 of each = 2 x 5 x 20mL = 200mL

Mark: 1 green line + 2 red lines

Stock Ery: 8mg/mL = 8000ug/mL

Make 100ug/mL working solution * 1,644 = 8000 * 0.0205?

20uL

into 1624uL

Using a 500mL flask: Assuming the uncertainty of surrological pipette negates the addition of volume by adding WS to the agar solution:

200mL * 0.01ug/mL = 100ug/mL * 0.02mL

160mL * 0.1ug/mL = 100ug/mL * (0.16 - 0.016 = 0.144)mL (as if already added 0.016mL = 160mL*0.01ug/mL/100ug/mL)

120mL * 0.5ug/mL = 100ug/mL * (0.60 - 0.12 = 0.48)mL

80mL * 1ug/mL = 100ug/mL * (1 - 0.4 = 0.6)mL

40mL * 2ug/mL = 100ug/mL * (0.8 - 0.4 = 0.4)mL

a. Starts at 200mL * 0ug/mL

1. Add 20uL WS
    a. 200mL * 0.01ug/mL
2. Remove 40mL for 2 plates at 0.01ug/mL
    a. 160mL * 0.01ug/mL
3. Add 144uL WS
    a. 160 * 0.1ug/mL
4. Remove 40mL for 2 plates at 0.1ug/mL
    a. 120mL * 0.1ug/mL
5. Add 480uL WS
    a. 120mL * 0.5ug/mL

6. Remove 40mL for 2 plates at 0.5ug/mL
    a. 800mL * 0.5ug/mL
7. Add 600uL WS
    a. 80mL * 1ug/mL
8. Remove 40mL for 2 plates at 1ug/mL
    a. 40mL * 1ug/mL
9. Add 400uL WS
    a. 40mL * 2ug/mL
10. Remove 40mL for 2 plates at 2ug/mL

08.10.16 control PCR reactions, pET26b transformation

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-08-10

WEDNESDAY, 8/10

Entry By: Tony

Purpose: To identify possible soures of contamination/nonspecific binding that may have been responsible for improper amplification of Las1 and Lux1 gBlocks; pET26b plasmids were also diluted to 40ng/uL and 80ng was retransformed into DH5a

Procedure:
The following samples were run in a ~25uL PCR reaction (12.5uL Q5 PCR mix + DNA/Primers + nuclease free H20)
(all DNA were diluted from original IDT DNA)

- Q5 PCR mix??
- Platinum PCR mix?
- 0.1ng Las1 gBlock
- 0.1ng Lux1 gBlock
- 0.25uM Common Forward Primer
- 0.25uM Common Reverse Primer
- 0.25uM Stitch Complement Primer (Las)
- 0.25mM Stitch Complement Primer (Lux)
- 0.1ng Las1 gBlock + 0.25uM Common Forward
- 0.1ng Lux1 gBlock + 0.25uM Common Forward
- 0.1ng Las1 gBlock + 0.25uM Common Reverse
- 0.1ng Lux1 gBlock + 0.25uM Common Reverse
- 0.1ng Las1 gBlock + 0.25uM Stitch Complement (Las)
- 0.1ng Lux1 gBlock + 0.25uM Stitch Complement (Lux)
- 0.1ng Las1 gBlock + 0.25uM Common Forward + 0.25uM Common Reverse
- 0.1ng Lux1 gBlock + 0.25uM Common Forward + 0.25uM Common Reverse

The following conditions were used. 25 cycles were run. Note that the new DNAP uses different anneal temps! See NEB Tm calculator
95C 3min initial melt
95C 30s melt
72C 30s anneal
72C 2min elongation
72C 5min final elongation
4C hold

08.30.16 IL-2/Cwa/Las1 Amplification

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-30

TUESDAY, 8/30

Plates were made:
MRS Ery agar at 0.25 (6x), 0.5ug/mL (5x)
LB Cm agar 0.133ug/mL

PCR of IL-2 and CWA2:
25uL of 2X PCR Mix
2.5uL F Primer
2.5uL R Primer
5uL of 0.05ng/uL DNA
15uL of ddH2O
50uL

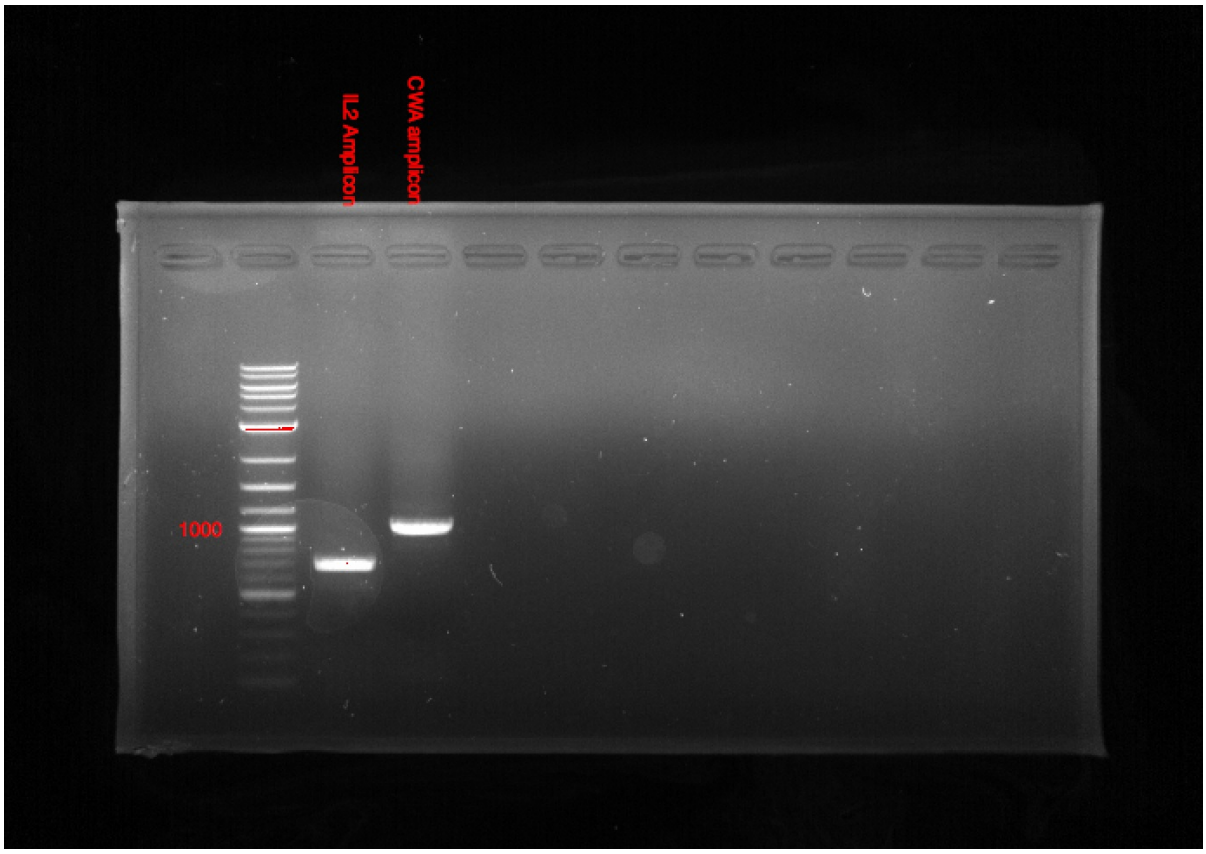
Stock: 10ng/uL

2uL * 10ng/uL = 0.05ng/uL * 400uL
So add 398uL ddH2O to 2uL of 10ng/uL CWA2 stock

9898727272
1.5min extension

5uL amplicon was run on 1% agarose
Both amplifications were successful

Screen Shot 2016-08-30 at 8.30.01 PM.png



Las1 was amplified in a 50uL reaction using previously successful protocol:
0.25mM Common Fwd new & Las1 Reverse new
0.2ng template DNA
Using conditions of 95, 72, 72C with 2min elongation for 25 cycles

08.29.16 PCR amplification of Las1

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

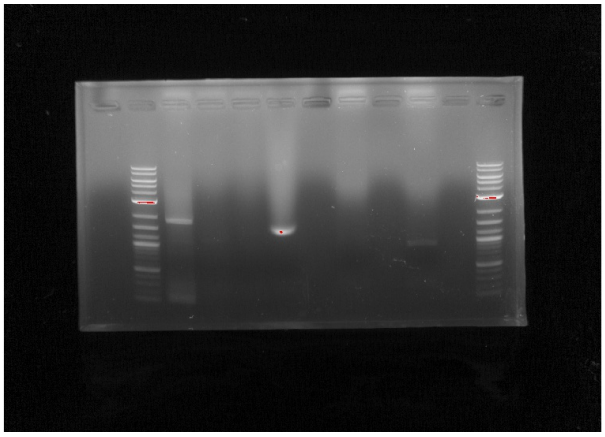
Date: 2016-08-30

TUESDAY, 8/30

Las1 was amplified in a 25uL reaction using Q5 mastermix
0.25mM Common Fwd new & Las1 Reverse new
2.5ng template DNA
Using conditions of 95, 72, 72C with 2min elongation for 25 cycles
Several controls were also run alongside Las1

Lanes: 2Log Ladder, Las1 amplicon (25uL), H2O, Q5 PCR mix, Platinum PCR mix
Amplification appeared to be successful.

Maxwell Ng 2016-08-30 12hr 37min.tif



Platinum PCR mix was discarded.

08.30.16 pIB184/CWA Digestions, Ligations

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-08-30

TUESDAY, 8/30

pIB184 and new Cwa samples were digested with EcoRI/PstI:

20uL =

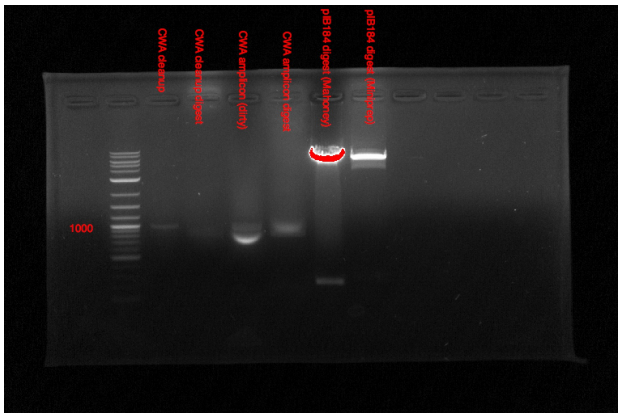
- 16uL DNA
- 2uL Cutsmart
- 1uL EcoRI-HF
- 1uL PstI-HF

Followed by incubation at 37C for 30m

The entire reaction was run on a gel alongside controls of undigested DNA

Digests appeared to be successful however the CWA cleanup digest was deemed of too low concentration to be usable

Screen Shot 2016-08-30 at 8.36.45 PM.png



CWA amplicon digest, and the 2 pIB184 digests were gel extracted using the kit protocol

The resulting gel extractions for CWA and pIB184 miniprep were ligated:

20uL =

- 1uL T4 Ligase
- 2uL 10X ligase buffer
- 10uL CWA DNA
- 5uL pIB184 DNA
- 2uL H2O

The reaction was incubated at room temperature overnight

Additional pIB184 was transformed into E.coli DH5a onto an Ery plate

pIB184 glycerol stock was grown in 8ug/mL Ery overnight

08.31.16 Electroporation pIB184-cwa2 (rough); Gel-Stitch PCR 6

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-31

WEDNESDAY, 8/31

Gel-Stitch PCR Protocol:
25uL PCR
5uL DNA
5uL DNA
15uL ddH20
= 50uL reaction
--> 25uL left after gel
    add 12.5uL PCR mix
    add 2.5uL of each Common primer
    add 12.5uL H2O

6th Stitch PCR Protocol:

1. Add 25uL of 2X PCR mix
2. Add 2uL Las gBlock 1 from 1/5 conc. PCR vial
3. Add 2uL Las gBlock 2 from 1/10 conc. PCR vial
4. Add 21uL ddH20
5. *Program the thermocycler to the procedure below:*
    a. Volume = 50uL
    b. 3:00 @ 94C (Hot Start? and dsDNA separation)
    c. 5 cycles:
        I. 0:30 (Denaturation) @ 98C
        II. 0:30 (Anneal) @ 40C
        III. 3:00 (Elongation) @ 72C
    d. FINAL: 5:00 (Elongation) @ 72C
    e. Hold 4C
6. Add 5uL of 2X PCR mix
    a. For Gel-Stitch, gel extract the ~3kb band size
7. Add 2.5uL of forward primer and 2.5uL of reverse primer (diluted eppendorfs)
    a. Volume = 55uL
    b. 3:00 @ 98C (Hot Start and dsDNA separation)
    c. 35 cycles:
        I. 0:30 (Denaturation) @ 98C
        II. 0:30 (Anneal) @ 72C
        III. 3:00 (Elongation) @ 72C
    d. FINAL: 5:00 (Elongation) @ 72C
    e. Hold 4C

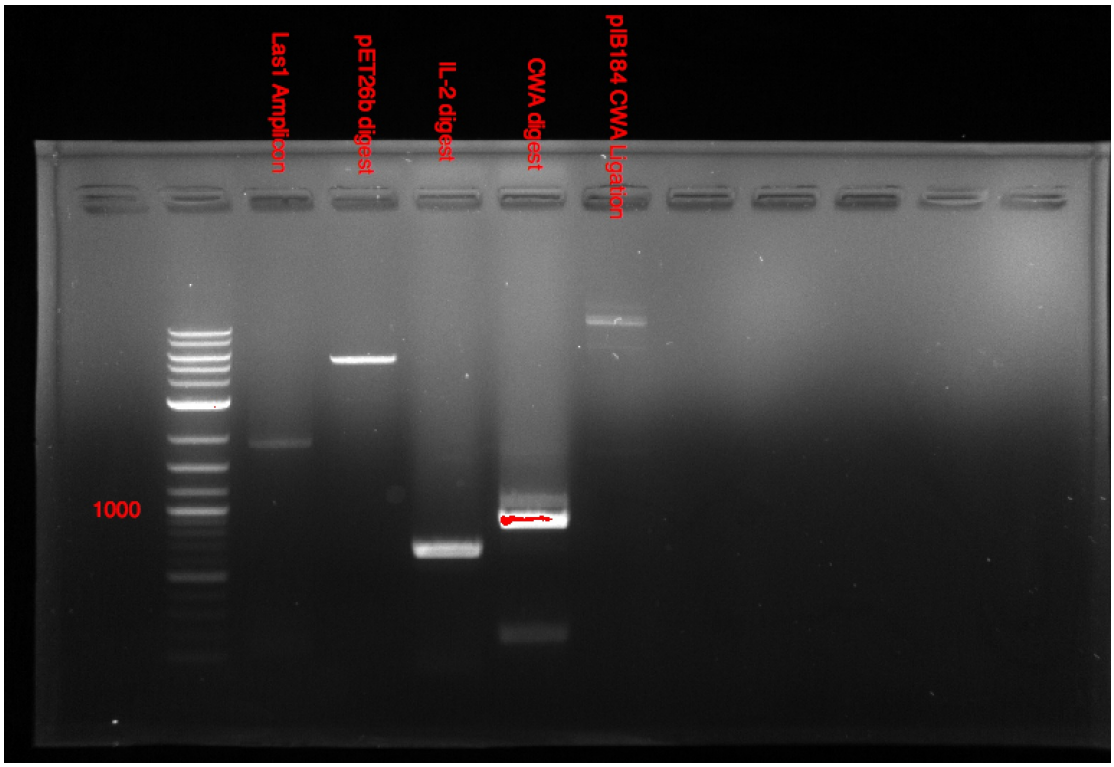
The incubator went to 47C, pIB plate discarded.

Note: There was no pIB184-mCherry left, so it was discarded

Digestions from previous day were run on a gel

Digestions appeared to be successful so were gel extracted
CWA digest resulted in 2 bands in the correct region so the bands were extracted separately to attempt ligation
pIB184-CWA ligation appeared successful so was electroporated into L.brevis (3uL) and plated onto 0.5ug/mL and 0.25ug/mL Ery MRS plates against a control

Screen Shot 2016-08-31 at 2.13.09 PM.png



08.31.16 Ligation of pET26b/IL-2, pIB184/cwa, pIB184 minipreps

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-08-31

WEDNESDAY, 8/31

A liquid culture from the pIB184 glycerol stock was miniprepped using the kit protocol.

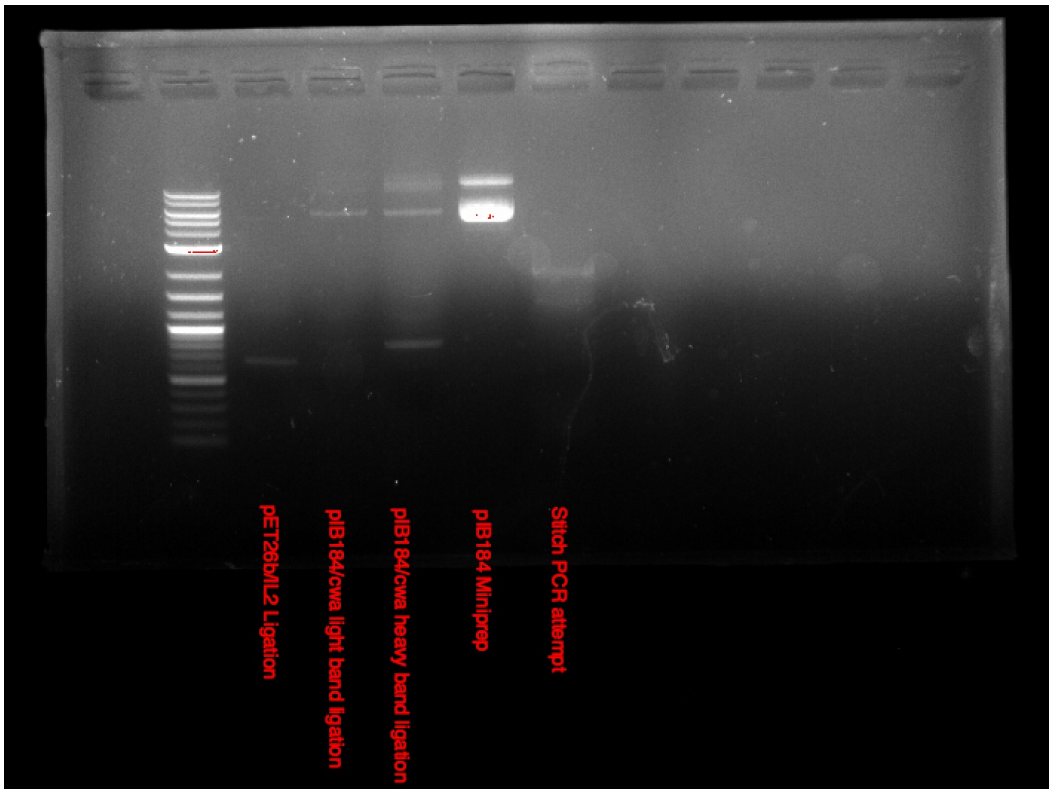
Ligations were performed on gel extracts of the digests in the previous entry.
Ligations were performed using 1uL T4 Ligase and made up to 20uL with nuclease free H2O
pET26b/IL-2: 8uL/8uL templates
pIB184/cwa light band: 1uL/3uL templates
pIB184/cwa heavy band: 4uL/7uL templates

Note: The digest of the miniprepped pIB184 was used to ligate

Samples were run on 1% agarose along with the miniprepped pIB184:
The concentrated PCR stitch reaction was also run: (see other Aug.31 entry)

pIB/cwa ligations appeared to be successful
pET26b/IL2 ligations are inconclusive; the pET26b band is faint and the ligated plasmid may be too low in concentration
pIB184 minipreps were successful
PCR stitch did not appear to be successful in creating a 3kb product.
0.25mM Common forward and Common reverse Primers were added to the reaction and cycled at 95, 40, 72 for 35 cycles

Screen Shot 2016-08-31 at 6.55.17 PM.png



09.01.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-09-01

THURSDAY, 9/1

Ligate pET26B with IL-2 redo
Transform pET26b/IL-2 + pIB184/CWA into E.coli
Gel electro check PCR results (if failed, reattempt stitch)

Glycerol stock of pIB184, (Miniprep)
Liquids of L.brevis if growth --> NO GROWTH, wait another 24hrs

Gel extraction liquidize of "pIB184 conc." using 50uL protocol

Digest pSB1C3 --> E * P

Mix:
5 ul NEB Buffer 2
0.5 ul BSA
0.5 ul EcoRI-HF
0.5 ul PstI
0.5ul DpnI
18.5 ul dH20

PCR IL-2, Las1, Las2

Plate

Gel:
Ligation diagnostic

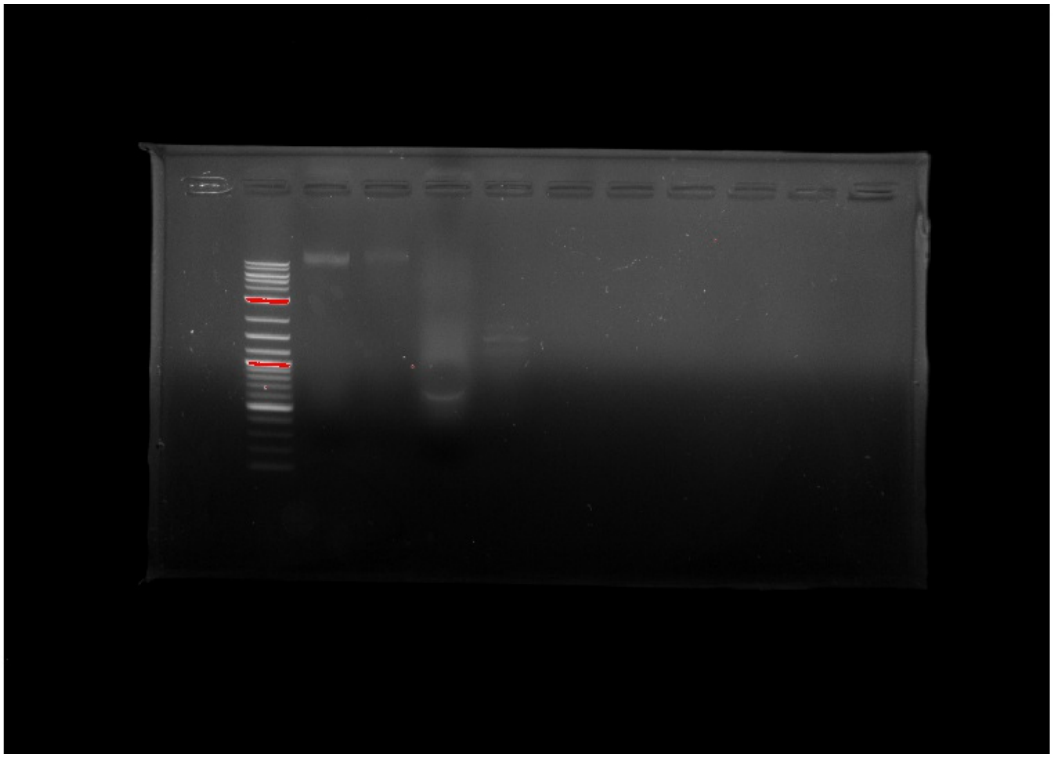
IL-2 Dig: = 12.5ng/uL
Add 250ng of DNA to be digested, and adjust with dH20 for a total volume of 16ul. = 8uL IL2 amplicon
Add 2.5ul of CutSmart
Make an added 2uL of BSA, E, P + 2uL H2O
then add 2uL of that to the reaction
There should be a total volume of 20ul. Mix well and spin down briefly.
Incubate the restriction digest at 37C for 30min, and then 80C for 20min to heat kill the enzymes. We incubate in a thermal cycler with a heated lid
Run a portion of the digest on a gel (8ul, 100ng), to check that both plasmid backbone and part length are accurate.

Ligation[adapted from iGEM, different]
Add 2ul of digested plasmid backbone (25 ng)
Add 1:3 molar amount of EcoRI-HF PstI-HF digested fragment (< 3 ul) = 25.3ng --> add 2uL of IL2 dig
Add 1 ul T4 DNA ligase buffer. Note: Do not use quick ligase
Add 0.5 ul T4 DNA ligase
Add water to 10 ul
Ligate 16C/30 min, heat kill 80C/20 min

Transform with 1-2 ul of product

Gel: (image lost)

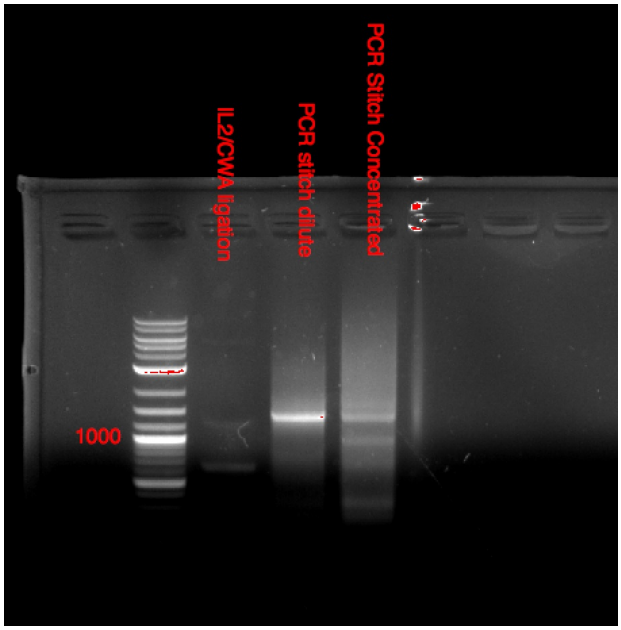
Maxwell_Ng_2016-09-09_19hr_33min.png



pIB184 (conc.) gel extract - concentrated successful
Failure of pET26b-IL2 ligation (was an IL2 dimer, likely due to the 1:3 molar ration of plasmid:insert - for future, use 1:1 molar ratio)

PCR results from previous day were run on 1% agarose as well as the attempted IL-2/CWA ligation

Screen Shot 2016-09-02 at 6.19.04 PM.png



09.02.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-09-02

FRIDAY, 9/2

Legend for plates:

2 black lines = kan

1 black kine = amp

1 red line = cm

2 red lines = ery

Previous pET26b-IL2 transformation was plated on the wrong plate (Cm)

No growth of pET26b-IL2 plates, pIB184-CWA heavy/light plates, or L. brevis pIB184-CWA or control plates in Dr. Surette's lab

Stitch PCR Attempt 7

Electroporation of pIB184

Plated on 0.25 and 0.5 ng/uL plates

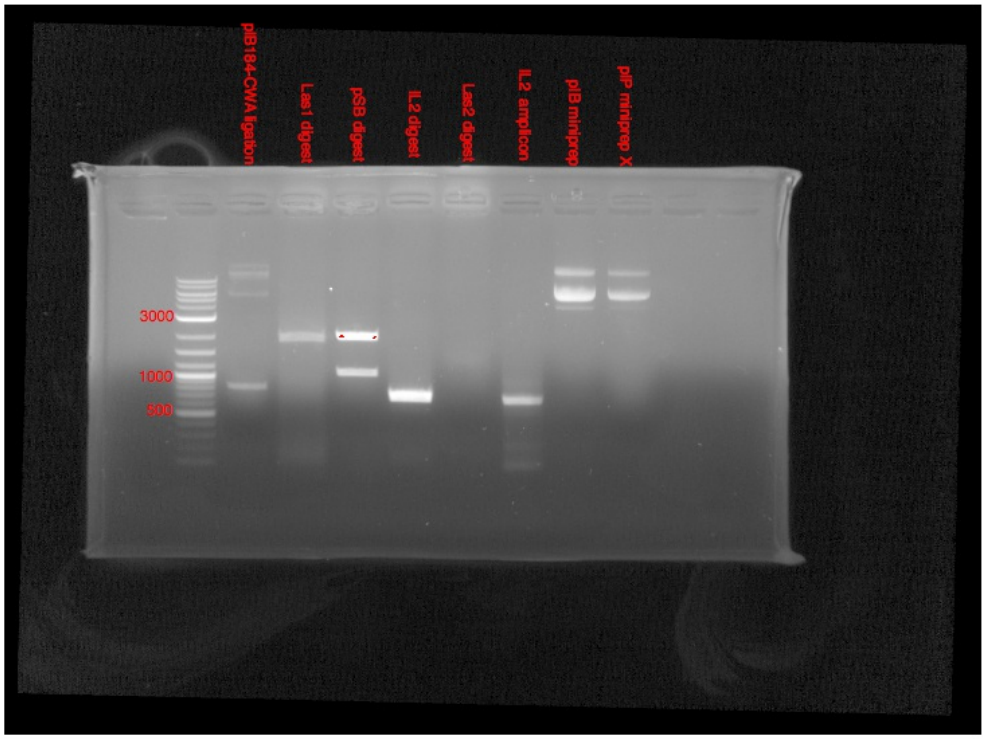
Transformation of pET26b-IL2, pIB184-CWA (heavy and light)

500uL SOC

1:30min incubate 37C

Gel: Ligations....PCR... pIB184 miniprep, pIB184 miniprep X (white precipitate from neutralization step may have contaminated)

new.tif


pIB184-CWA ligation
Las1 digest
pSB digest
IL2 digest
Las2 digest
IL2 amplicon
pIB miniprep
pIP miniprep X
3000
1000
500

09.03.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-09-03

SATURDAY, 9/3

The incubator was set to 30C-33C; little to no growth on plates, but could be slow. Some may have tiny colonies.

1. Liquid culture of a tiny possible colony on pET26B-IL-2 (Kan)
    a. Cm/Kan/Amp plates:
    b. Split 500 mL bottle into ~350 mL and ~150mL kanamycin and chloramphenicol, respectively.
        I. Added 40 uL kanamycin (100ug/uL stock in ddH2O, -20C) to ~350 mL LB agar
        II. So ~11uL/mL

40uL * 100ug/uL / 350mL = 11.4ug/mL --> 11ug/mL

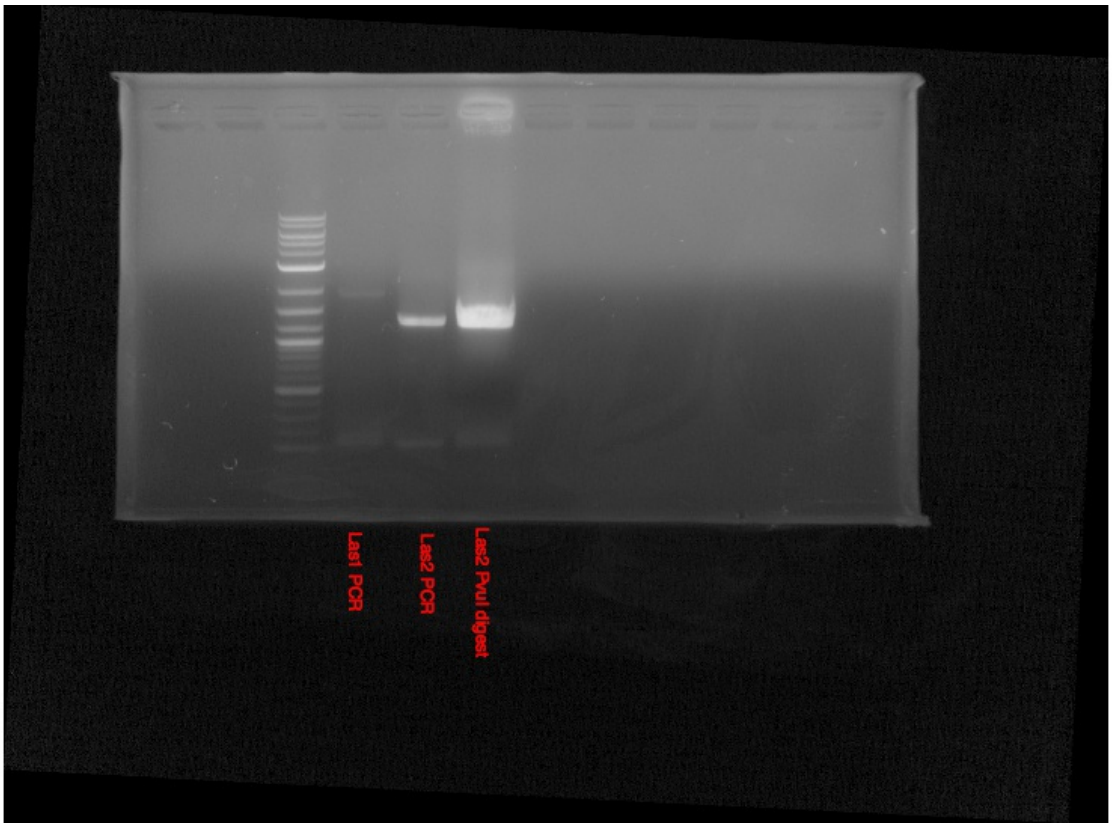
11ug/mL x 5mL = 100000ug/mL x 0.00055mL = 0.55uL

2. Digest Las2 for ligation with Las1 (PvuI)

3. Make a gel

4. Run a gel on Las1, Las2 PCR amplicons, Las2 digest

las1las2las2digest.png



5. Gel extract Las1 digest, pSB digest, IL2 digest, and the new Las2 digest

6. Ligate overnight Las1 with Las2 ; ligate overnight pSB with IL2 (Cm)
10u Las1 7.5u Las2
3u IL2 7u pSB

---Future---
7. Run diagnostic gel on Las1-Las2 and pSB-IL2

8. Transform pSB-IL2 into gel --> Others must try for different technique

9. Digest Las1-Las2 with E & P, ligate into plasmids pSB1C3 (for submission, E. coli growth/miniprep) and pIB184 (for L. brevis / E. coli), transform respectively

---mCherry-IL2---

1. Digest mCherry with SptI and EcoRI
2. Digest IL2 with XbaI and XhoI AND Digest IL2 with XbaI and PstI
3. Ligate construct E-mCherry-M-IL2-XhoI (for pET26b) and E-mCherry-M-IL2-P (for pSB1C3, for submission)
4. Transform in E. coli

Shopping list:
G+ miniprep kit?
Ni columns
IL2 assay - if DH5a cells of pET26b-IL2 grows (Yosef)
Eppendorfs (1.5mL)
Gloves (S, M, L)

09.09.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Tony Chen

Date: 2016-09-09

FRIDAY, 9/9

100ug/mL x VmL = 0.5ug/mL x 5mL

For 5mL of liquid culture, use 25uL of the NEW Ery stock (of 100ug/mL).

Liquid cultures of the 0.25 and 0.5 Ery MRS CWA plates, at 5mL

Transformations of

1/30 dilution

10.03.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-10-03

MONDAY, 10/3

There was a spill when I arrived at ~8pm

Cm 0.14

8mL x 0.13 mg/mL = 50mg/mL x V
V = 0.0208mL = 20.8uL

October 8, 2016 Entry

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-10-08

SATURDAY, 10/8

Make gel for 3 digests

Plates pIB184/pIB184-CWA and IL-2 worked; no growth on NiCo promoter

Liquid ery DH5a pIB184 and pIB184-CWA

Liquid cm DH5a IL-2

Transformation NiCo

Ery liquids = 5mL
8ng/uL = 8ug/mL =

5mL * 8ug/mL = V * 100ug/mL
V = 0.4mL
400uL of Ery [100ug/mL] stock
4.6mL of LB

Cm liquids
50mg/mL = 50'000ug/mL
--> 1ug/mL

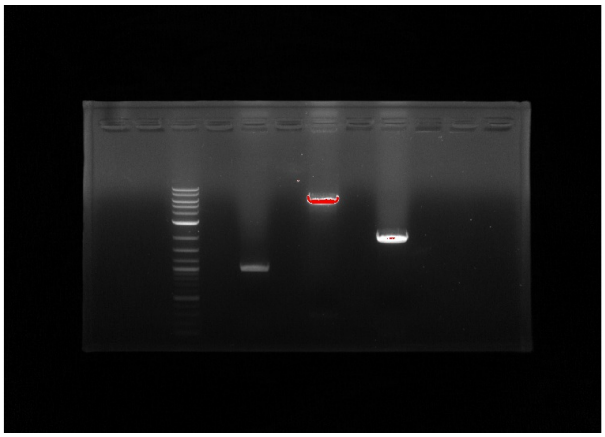
50ug/mL * V = 50'000ug/mL * 0.002mL
V = 2mL

V = 10mL stock, using 20uL crazy stock

0.13ug/mL * 3mL = 50ug/mL * V

V = 0.0078mL = 7.8uL (2.6uL per mL)

Maxwell Ng 2016-10-08 18hr 08min.png



10.11.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-10-11

TUESDAY, 10/11

NiCo Plate grew over 2 days ~34C

pSB-IL2 did not grow on plate, but grew in liquid (contamiation? or single colony?)

pIB/pIB-CWA did not grow in liquid
Started a new picked liquid culture
+4C fridged pIB plate will now go into the ~34C incubator for additional growth

3 digests results:
mCherry still doesn't work, CWA worked (no more CWA, needs to be PCRd if want non-digested), pSB1C3 worked - cut out at ~2kb

----Night plan----

1mL into 3mL new liquid of pSB-IL2 total 4mL
1mL to a 2mL glycerol of pSB-IL2
1mL to miniprep

5mL liquid of NiCo
5mL * 0.13ug/mL = V * 50ug/mL
V = 13uL

5mL liquid of pIB184-CWA

Incubator pIB184-CWA and pSB-IL2

Gel extract CWA dig. and pSB dig.
psb = 0.097g
cwa = 0.112g

----Future----
PCR CWA

Ligation of pSB & CWA together

Transformation of pSB-CWA
Liquid pSB-CWA
Miniprep pSB-CWA

Submission kit and send of pSB-IL2 and pSB-CWA

Make a new kind of RFP

10.12.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Maxwell Ng

Date: 2016-10-12

WEDNESDAY, 10/12

The ELISA of IL-2 did not work, so we switch our validation piece to CWA

WEDNESDAY

Status of liquids? NiCo

--> Tony did a bunch of things

PCR 50uL of CWA

Diagnostic gel of pSB-IL2 DNA

- Max miniprep did not work, Tony miniprep worked
- NiCo miniprep worked

Ligation of pet26b and CWA together

Ligation of pSB and CWA together

- pSB = 2kb, CWA = 1kb
- want 3insert:1vector
- similar densities on the gel image (image lost to computer reboot, mCherry failed again to digest)

2uL 10x ligation buffer

5uL pSB

7.5uL CWA

4.5uL ddH2O

1uL T4 ligase

PCR cleanup of CWA

Dhany

Hydrate new kit RFP DNA = http://parts.igem.org/Part:BBa_K516030

Transformation of pet26b-CWA into BL21

Transformation of pSB-CWA into DH5a

Plate/Liquid pet26b-CWA

Plate/Liquid pSB-CWA

THURSDAY

Rayu morning at Dr. Surette's with Michelle: Electro-transformation of L.brevis with pIB184-CWA

Miniprep pSB1C3-CWA

Digest pet26b with RE: _____

Digest CWA with RE: _____

---> Upon analysis, nothing seems to fit
Upon analysis, I don't see any way to get the acmA1 cwa gene into pET26b (NdeI, XbaI, and EcoRI are all on the prefix side and nothing I saw is on the suffix side of our GOI)

https://benchling.com/vivianlau/f/a3cbADt3-cell-wall-anchor-fusions/seq-TwkitCyH-acma1-cwa-6his-her2-darpin/edit
https://www.addgene.org/browse/sequence_vdb/2563/

Except for the SpeI and XbaI scarring

SATURDAY
Give Karanbir the pet26b-CWA bacteria

Submission kit and send of pSB-IL2 and pSB-CWA

10.13.16

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-10-13

THURSDAY, 10/13

THURSDAY

Rayu morning at Dr. Surette's with Michelle: Electro-transformation of L.brevis with pIB184-CWA

---> Problem: no pIB184-CWA DNA - going to re-attempt the previously empty miniprep from a fridge liquid - will attempt gel on Friday. Re-grow a glycerol stock of pIB184-CWA E.coli DH5a in liquid culture - miniprep if growth and gel on Friday if previous miniprep fails.

Miniprep pSB1C3-CWA (cannot miniprep - was not grown in liquid - very few potential colonies but attemped culture)

Growth of plate with pSB-RFP and pSB-CWA? (very little growth, may not be actual colonies)

---> Liquids

CWA PCR cleanup?

Digest pet26b with RE: XbaI

Digest CWA with RE: XbaI & SpeI

Ligate together

Transform

Plate onto Kanamycin plate

FRIDAY

Make liquid culture of pIB184-CWA

SATURDAY

Give Karanbir the pet26b-CWA bacteria

Submission kit and send of pSB-IL2 and pSB-CWA

10.14.16 Gel of pIB-CWA miniprep and pSB-IL2 miniprep, Miniprep 2-18D (mCherry) culture

Made with Benchling

Project: McMaster iGEM '16

Authors: Chirayu Bhatt

Date: 2016-10-14

FRIDAY, 10/14

Entry by: Rayu Bhatt

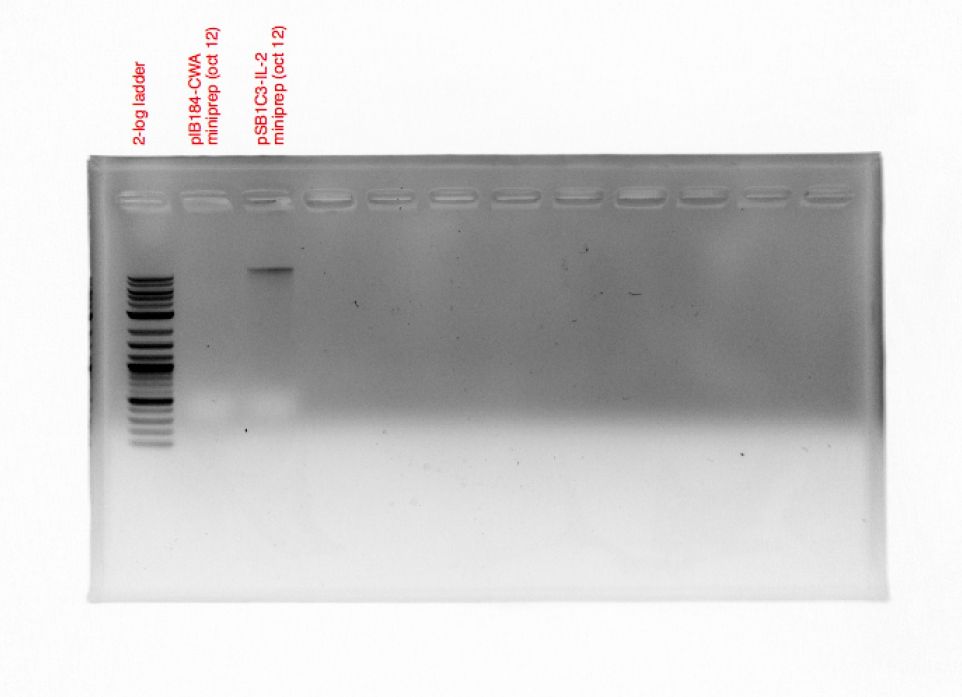
Experiments:

- Re-attempting a gel of pIB-cwa E. coli miniprep from October 12
- Attempting gel of pSB-IL2 E. coli miniprep from October 12
- Electroporation (Not attempted due to lack of usable DNA)
- Re-diluting successful liquid cultures of BL21 cells (control and pET26b-IL-2)
- Miniprepping mCherry glycerol stock culture from October 12

Results:

- October 13 liquid cultures had no growth for Erythromycin cultures
    - No growth in pIB-cwa E. coli from plate colonies (on split plate)
    - No growth in pIB-cwa E. coli glycerol stock re-culture
- Gel Electrophoresis using 1% Agarose gel (1: ladder, 2: pIB-cwa miniprep (TC), 3: pSB-IL2 miniprep)
    - 10uL of ladder
    - 10uL of each miniprep with 2uL of 6x loading dye added to achieve final volume of 12uL

Screen Shot 2016-10-14 at 12.35.15 PM.png



    - No yield for old CWA miniprep - confirmed failure
- mCherry miniprep is in -20. Please validate this miniprep in the future.
- GLYECEROL STOCK OF pIB184-CWA STILL NEEDS TO BE LIQUID CULTURED WITH Cm TO SEE IF LABELLING ERROR WAS THE CAUSE OF LACK OF GROWTH (where the actual label was pSB1C3-CWA)